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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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CONTENTS OF VOLUME XX.

NO. 1. DECEMBER, 1910.

CHILD, C. M. *Further Experiments on Adventitious Reproduction and Polarity in Harenactis* 1

PEEBLES, FLORENCE. *On the Interchange of the Limbs of the Chick by Transplantations* .... 14 ✓

BROWNE, ETHEL N. *The Relation Between Chromosome Number and Species in Notonecta* 19

GOODALE, H. D. *Some Results of Castration in Ducks* 35

TOWER, WM. L. *The Determination of Dominance and the Modification of Behavior in Alternative (Mendelian) Inheritance, by Conditions Surrounding or Incident upon the Germ Cells at Fertilization* 67

NO. 2. JANUARY, 1911.

MONTGOMERY, THOS. H., JR. *Certain Habits, Particularly Light Reactions, of a Littoral Aranead* .... 71 ✓

ISELY, F. B. *Preliminary Note on the Ecology of the Early Juvenile Life of the Unionidæ* 77

CARY, LEWIS R. *A Study of Pedal Laceration in Actinians* .... 81 ✓

STEVENS, N. M. *Further Studies on Heterochromosomes in Mosquitoes.* 109 ✓

STEVENS, N. M. *Preliminary Note on Heterochromosomes in the Guinea Pig* .... 121 ✓

NO. 3. FEBRUARY, 1911.

RICHARDS, A. *The Method of Cell Division in the Development of the Female Sex Organs of Moniezia* .... 123 ✓

COKER, ROBERT E., AND SURBER, THADDEUS. *A Note on the Metamorphosis of the Mussel Lampsilis lævissimus* .... 179 ✓

McINDOO, NORMAN E. *Notes on Some Arachnids from Ohio Valley Caves* .... 183 ✓

HARGITT, CHARLES W. *A Further Note on Keratosum complexum* 187 ✓

NO. 4. MARCH, 1911.

SMITH, BERTRAM G. *The Nests and Larvæ of Necturus* .... 191 ✓

DANFORTH, C. H. *A 74 mm. Polyodon* 201

KING, HELEN DEAN. *Studies on Sex Determination in Amphibians, IV* 205 ✓

No. 5. April, 1911.

Hegner, Robert W. *Experiments with Chrysomelid Beetles. III. The Effects of Killing Parts of the Eggs of Leptinotarsa decemlineata* .... 237 ✓

Scott, John W. *Further Experiments on the Methods of Egg-laying in Amphitrite* .... 252 ✓

Kepner, William A. *Nematocysts of Microstoma* 266

Tanquary, Maurice C. *Experiments on the Adoption of Lasius, Formica and Polyergus Queens by Colonies of Alien Species* .... ~~282~~ 281

No. 6. May, 1911.

Child, C. M. *Experimental Control of Morphogenesis in the Regulation of Planaria* 309

Cochran, M. Ethel. *The Biology of the Red-backed Salamander (Plethodon cinereus erythronotus Green)* 332

Osborn, Henry L. *On the Distribution and Mode of Occurrence in the United States and Canada of Clinostomum marginatum, a Trematode Parasite in Fish, Frogs and Birds* 350

Cockerell, T. D. A. *The Scales of Freshwater Fishes* 367

Vol. XX. *December, 1910.* *No. 1*

BIOLOGICAL BULLETIN

FURTHER EXPERIMENTS ON ADVENTITIOUS REPRODUCTION AND POLARITY IN HARENACTIS.

C. M. CHILD.

WITH ELEVEN FIGURES.

INTRODUCTORY.

In a paper which appeared in 1909[1] I described the adventitious formation of new axes in the Californian actinian, *Harenactis attenuata*. These new axes have thus far been observed only under certain conditions, viz., in isolated pieces prepared in such manner that the oral cut surface of the body wall unites with the aboral about the whole circumference. In my earlier paper such pieces were designated as "rings."

During the past summer I again enjoyed the privileges of the laboratory at La Jolla and take this opportunity of expressing my thanks to the director, Professor Ritter, and to the trustees of the San Diego Marine Biological Association. During my stay at La Jolla I obtained results with "rings" from *Harenactis* which supplement my earlier work and extend and confirm my conclusions. These results form the subject of the present paper.

As described in my earlier paper, the rings are produced by isolating rather short, cylindrical pieces from the body of *Harenactis* by means of transverse cuts (Fig. 1), and then removing more or less completely from these pieces the mesenteries and mesenterial muscles. The region of the body proximal to the esophagus is the most suitable for such operations, since here the mesenteries are well developed, bear large retractor muscles and extend into the enteron with free borders instead of being attached to the esophagus, as in the more distal regions, or being only slightly developed, as in the more proximal, attenuated region.

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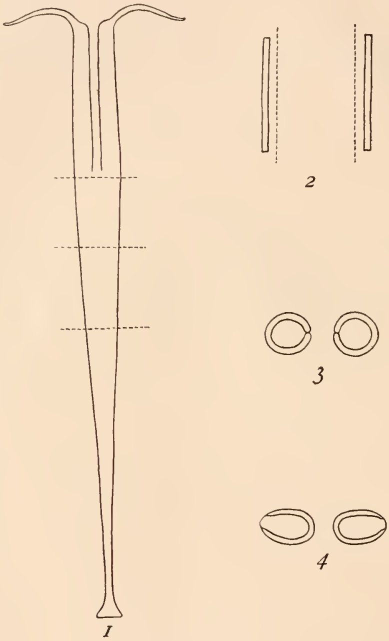
[1] Child, '09*b*, "Factors of Form Regulation in *Harenactis attenuata*, III., Regulation in 'Rings,'" *Journ. Exp. Zool.*, VII 2, 1909.

The removal of the free borders brings about longitudinal contraction of the injured mesenteries and so approximates the oral and aboral cut surfaces of the body wall. This contraction is merely a special case of the wound contraction so characteristic of the actinians, which I have described for *Cerianthus* and *Harenactis*.[1] In my earlier experiments I removed merely such



Figs. 1–4.

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[1] Child, '03, "Form Regulation in *Cerianthus*, I., The Typical Course of Regeneration," Biol. Bull., V., 5, 1903.

'04, "Form Regulation in *Cerianthus* III., The Initiation of Regeneration," Biol. Bull., VI., 2, 1904.

'08, "Form Regulation in *Cerianthus æstuari*," Biol. Bull., XV., 1, 1908.

portions of the mesenteries as protruded from the cut ends of the pieces, or accomplished the same result by other means.[1] In the experiments of this summer greater care was taken to make certain that all the muscles were removed, and the free borders of the mesenteries—in most cases the greater part of the mesentery as well—were cut away. After these operations the pieces consisted of the body wall with only the bases of the mesenteries, *i. e.*, those portions which lie nearest the line of attachment to the body wall. The nature of this operation is indicated approximately in the diagrammatic Fig. 2. This represents a typical longitudinal section of the body wall of the cylindrical piece: all the internal mesenterial structures between the two broken lines are removed, leaving only narrow strips of mesenterial tissue next to the body wall.

Such pieces form rings in almost every case, and nearly all of the rings give rise to tentacle groups. The result of the removal of the mesenteries is the approximation and contact of the oral and aboral cut ends of the body wall, as indicated in the optical section, Fig. 3. Union occurs between these cut surfaces and the body wall thus forms a closed ring with an opening through the center and with no connection between the enteron and the exterior. The enteric cavity becomes more or less distended with water after closure, undoubtedly in consequence of the passage of water through the body wall, and sooner or later a peculiar rotation of the parts about a circular axis situated in the enteric cavity of the ring occurs, as described in my earlier paper ('09*b*, pp. 356–357). The result of this rotation is a change in the position of the line of union between the oral and aboral ends from its original position around the central opening to the outer surface of the ring (cf. Figs. 3 and 4 of the present paper; in Fig. 4 the region of union between oral and aboral ends is indicated by the very thin body wall which is formed of new tissue). Very commonly the line of union, which is marked by the formation of more or less new tissue, finally comes to occupy a position somewhat upon the upper surface of the ring as this

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'09*a*, "Factors of Form Regulation in *Harenactis attenuata*, I., Wound Reaction and Restitution in General and the Regional Factors in Oral Restitution," *Journ. Exp. Zool.*, VI., 4, 1909.

[1] Child, '09*b*, Figs. 2–5, Fig. 31, also pp. 373–374.

lies on the bottom of the dish (Fig. 4). If the ring is turned over, a new rotation often occurs, which brings the line of union into approximately the same relative position. As stated in my earlier paper ('09*b*, p. 357), this appears to be an "attempt" at orientation with respect to the substratum, but since the original distal and proximal ends of the piece are united with each other and since, as is evident from the later processes in these pieces, the original polarity is decreased or almost eliminated by this union, any complete orientation is of course impossible.

Most of these rings give rise in the course of several weeks to groups of tentacles or single tentacles which lie along the line of union, but which may arise wholly on one side or the other, *i. e.*, wholly from the original distal or the original proximal parts, or may involve both in the formation of a single tentacle group.

In some cases these groups of tentacles are without any close resemblance to the normal disc of *Harenactis* and they often show a more or less marked bilateral or biradial symmetry with respect to the line of union (Child, '09*b*, Fig. 10). More commonly, however, each group shows a more or less distinct radial symmetry, similar except as regards the number of radii, to the symmetry of the animal in nature (Child, 09*b*, Figs. 12, 15, 17–30). In these radially symmetrical tentacle groups mesenteries could in many cases be seen distinctly upon the small discs, their arrangement corresponding with the intervals between the tentacles. In some cases these were clearly continuations of the old mesenteries (Child, '09*b*, Figs. 18–20), but in other cases I was unable to find any such connection and was uncertain whether the formation of new mesenteries had occurred or not.

In my earlier experiments the largest number of tentacles in a radially symmetrical group was seven ('09*b*, Fig. 29*e*) and in no case were mouth and esophagus present, though in one ring several groups showed a tentacle-like outgrowth in the center of the disc, where the mouth would normally appear. I suggested that this outgrowth might possibly represent an everted esophagus, but could not be certain as to its nature.

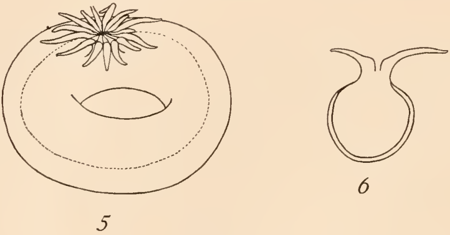
These tentacle groups were interpreted as representing new polarities, new body-axes, developing adventitiously in the region of union of the original distal and proximal ends of the piece, but failing to attain the usual form because of the peculiar conditions under which they arose.

In returning to these experiments this summer, I hoped to obtain tentacle groups which should approach more closely the usual form of *Harenactis* and thus leave no doubt that these outgrowths really represent new polarities, in short, that these phenomena in rings are really a form of asexual reproduction related to certain cases of the formation of adventitious structures in plants. These hopes were fully realized in one case, to be described below, and other tentacle groups among the large number obtained showed various points of interest.

Description of Experiments.

The case of greatest interest is one in which only one group of tentacles appeared upon the whole ring. This group was situated wholly upon one side—probably the oral—of the line of union, was radially symmetrical from the beginning and at first possessed seven or eight tentacles. During its growth, however, new tentacles appeared until the number reached sixteen. The tentacles were regularly arranged about a disc similar in appearance to that of the normal animal and in the center of the disc a mouth, opening into a short esophagus, appeared. On the disc sixteen mesenteries could be counted, most or perhaps all of these being new, as was evident both from their appearance and relation to other parts and from the impossibility that sixteen of the twenty-four old mesenteries should be involved in this new development from a small area localized on one side of the ring. Fig. 5 shows this new disc with the sixteen tentacles as it appeared twenty days after the operation. The line of union between the original oral and aboral ends of the piece is indicated by the dotted line. This new individual differs from the full grown animal in nature in the smaller number and unequal length of the tentacles and in the absence of a proximal end, but there can be no doubt that it represents a new body-axis, a new polarity.

After the formation of the disc it gradually became elevated above the surface of the ring by the growth of a cylindrical column beneath it. Fig. 6 is a diagrammatic optical section through the side of the ring on which the disc appeared; it shows the formation of the column beneath the disc. After twenty-five days the old tissues of the ring began to show signs of disintegration and the new individual was either poisoned or infected by the dead and putrefying tissue, and died. In no case thus far has it been possible to keep these outgrowths of the rings permanently alive, for the parts not involved in the new outgrowths always degenerate sooner or later and finally kill the new parts. In the case just described it was my intention to separate the new individual from the ring in order that it might if possible complete its development, but death occurred before this was done. If the opportunity of still further experiment arises, I shall try the experiment of separating some of these adventitious buds, for they are such, from other parts of the ring. I have no doubt that under proper conditions they can be kept alive and fed so that development will proceed.

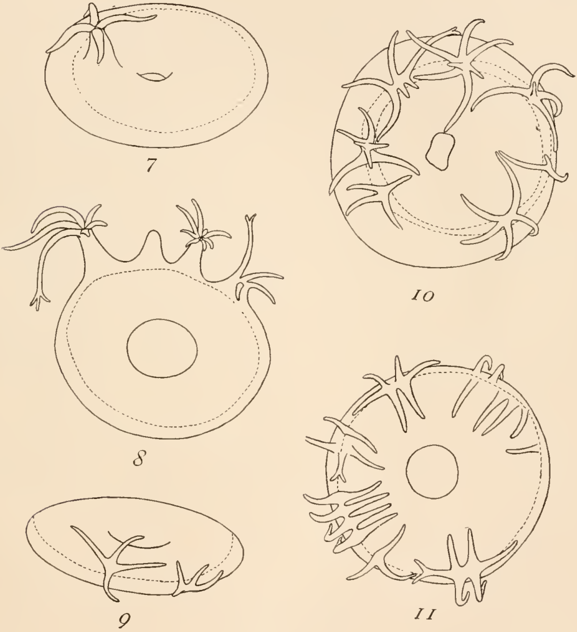


FIGS. 5–6.

This case as it stands is, however, sufficient to demonstrate that new polarities may arise from these rings, from which the old polarity is in large measure eliminated. As was pointed out in my earlier paper, this is a form of reproduction which resembles more or less closely certain cases of the formation of adventitious structures in plants. Moreover, there can be no doubt that each new polarity arises in relation to local conditions in the growing tissues along the line of union between the original oral and aboral ends of the piece, though exactly what these conditions are, we do not at present know.

In the course of my experiments a large number of other adventitious tentacle groups was obtained, many of them with new mesenteries, but none with so large a number of tentacles as in the above case and none with mouth and esophagus. Besides the distinctly radially symmetrical groups, others, showing the most various degrees of radial and bilateral symmetry and asymmetry, appeared. Brief descriptions and figures of a few characteristic cases are added here.

Fig. 7 shows a case of considerable interest: here a disc with four radially arranged tentacles and with four well-developed mesenteries, but no mouth, appeared on one side of the line of union and was gradually elevated by the growth of a long column beneath it. No further tentacles were added during the life of this specimen and it finally died, apparently from the same causes as others.



FIGS. 7–11.

In Fig. 8, a ring with three tentacle-bearing outgrowths and one without tentacles is shown. The tentacles on two of the three outgrowths are radially symmetrical, though unequal in length and the discs show well-developed, radially arranged mesenteries, but are without mouths. The third group is less symmetrical and consists of only four tentacles. All the outgrowths appeared on one side, apparently the oral, of the line of union.

Fig. 9 shows a case, viewed obliquely from one side, in which two groups of four tentacles each appeared, one on either side of the line of union. This is the only case observed thus far in which distinct groups appeared on different sides of the line of union in a single ring and it is figured on that account.

A case with seven distinct tentacle groups is shown in Fig. 10. This is the largest number of distinct groups observed in any single case. Apparently the formation of tentacle groups is in general closely connected with the growth of new tissue in the region of union, for they appear to be localized at points where the areas of new tissue are greatest. At some points the two cut edges may unite with scarcely any new tissue between them, and in such regions tentacles are less likely to appear than in regions with more new tissue. In the case shown in Fig. 10 an unusually large amount of new tissue appeared as a more or less continuous band about most of the circumference and in this the tentacle groups developed. The area of the new tissue is approximately indicated in the figure by the two dotted lines.

And finally, Fig. 11 shows a case in which the symmetry of the groups is mostly bilateral rather than radial. Here the corresponding parts of each group arise on opposite sides of the line of union. I believe that groups of this kind appear when the old organization is less injured and the old polarity less completely eliminated than in other cases. Although tentacles appear in such cases on the aboral as well as on the oral side of the line of union, nevertheless the development of such rows of tentacles is manifestly a less extreme change from the "normal" arrangement with respect to the cut surface of the body wall than the formation of distinct, radially symmetrical groups on one side or the other of the line of union. It will be observed from the figure that in two cases the tentacles which apparently arise directly upon the line of union are forked at their tips: this means that they began their development as two separate tentacles, one on each side of the line of union, and later united to form a single tentacle. Similar conditions are shown in Fig. 8, *b*, and in Fig. 10 of my earlier paper ('09*b*).

CONCLUSION.

As regards the interpretation of these peculiar structures, there is little to add except by way of confirmation to the conclusions of my preceding paper on the subject. The outgrowths on the rings unquestionably represent a kind of reproduction which results from a certain degree of physiological isolation of parts, in other words from the decrease in physiological correlation, which is itself the result of the removal of important elements of the original organization and of the union of the oral with the aboral end. Moreover, it seems evident that the localization of the tentacle groups along the line of union is due to "chance" factors, probably in part to differences in the amount and rapidity of the growth of new tissues and on the other hand to differences in the degree of injury to the old organization.

That the new polarities have no direct relation to the original polarity is evident from the fact that they may arise from either the oral or the aboral side of the line of union, or from both sides. From these facts we must conclude that they are the result of local conditions in the regions concerned, and of conditions which are not primarily concerned with the original organization.

As regards symmetry, the new growth may be either bilaterally or radially symmetrical or asymmetrical. Clearly the symmetry, as well as the polarity, has nothing to do with the original organization.

And finally, these regulatory phenomena cannot be satisfactorily interpreted by the hypothesis which regards polarity and symmetry as essentially a summation of the polarities and symmetries of ultimate oriented particles or molecules. How, for example, shall we account on the basis of this hypothesis for the appearance of bilaterally symmetrical groups of tentacles in an organism which is naturally radially symmetrical. Furthermore, how, unless we accept Driesch's entelechy, can we account for the complex changes in orientation of the particles which must occur in the development of a new polarity and radial symmetry from a small area of the oral or aboral end of the piece. If we turn to the process of crystallization for assistance, we meet difficulties, for we should expect, if the process of organic development is analogous to crystallization, that *Harenactis* material would in general conform to a particular type of arrangement, at least within certain limits of environmental change.

On the other hand, if we regard polarity and symmetry as molar phenomena resulting from the localization by any conditions or agents of certain metabolic processes differing in degree or kind, together with the physiological correlation resulting from such localization, we can readily understand how new polarities may arise without reference to the old, in response to local factors, and how bilateral symmetry may appear in one case and radial in another in the same organism.

The greater the extent to which we interfere with or destroy the old polarity and symmetry by removing or altering the localization of the original metabolic processes, the greater the possibility of the origin of new polarities and symmetries in response to local conditions.

In the recent experiments of Lillie, Morgan and others on the effects of centrifuging eggs, the polarity and symmetry of the eggs are, at least in certain cases, apparently not altered by the displacement of the visible granules. These results have led various experimental embryologists to adopt the hypothesis of orientation of molecules or particles as the basis of polarity and symmetry. As a matter of fact, however, the visible granules which are displaced in these experiments are merely the inactive or relatively inactive products of metabolism and so long as they are visible, have reference primarily to past activities. Their localization, in so far as they are localized with respect to an axis, suggests the more or less sharp localization along this axis of metabolic processes differing in degree or kind. There is no reason to believe that displacement of the granules by centrifuging alters essentially the localization of the processes, consequently the polarity and symmetry of the egg may remain unchanged, whatever the positions of the visible granules. As a matter of fact most, if not all eggs actually show a more or less sharp localization along the polar axis of processes differing in degree or kind: in general the region of the animal pole reacts more rapidly in various ways than the vegetative pole and between these two poles a gradation apparently exists. In every fragment of such eggs above a given size this gradation must exist in some degree, *i. e.*, such fragments will retain the original polarity of the whole. If, however, we could control the size of isolated egg fragments and if it were possible to reduce the size of nucleated fragments indefinitely we should undoubtedly find a limit below which polarity would no longer be apparent. In every case where control of the size of isolated pieces is possible, such a limit has been found: unfortunately the egg cell is exceedingly unfavorable material for experiment along this line. It is at present extremely difficult and often impossible to isolate pieces of the egg of a certain desired size or from a certain desired region: moreover, if the isolated fragment is to live and show any developmental processes it must possess a nucleus. Manifestly the possibilities of investigating the polarity and symmetry of the egg by means of isolated fragments are very narrowly limited, as compared with the possibilities which simple organisms with elongated axes present, and we have no right to draw conclusions as to the nature of polarity from experimentation on eggs alone. Until we can actually prove that in eggs polarity does not decrease with decrease in size below a certain limit, or until we can demonstrate the orientation of particles or molecules by means of the polariscope, we have no adequate grounds for regarding polarity as anything but a molar phenomenon. Moreover, the fact already mentioned, that in all cases where control of the size of the isolated piece is possible, and where extended experimentation has been made, the phenomena of polarity do decrease with decreasing size, should suggest the necessity of caution in drawing conclusions from eggs alone, where both technical and natural obstacles to exact experimentation along these lines exist. Moreover, I fail to see how we can avoid accepting the evidence which the polariscope gives as possessing much greater weight than the results of experiment with our present crude technique, and this evidence is, except for certain highly specialized structures, *e. g.*, the nerve fiber, negative, so far as I am aware. If an orientation of particles is the basis of organic polarity and symmetry, surely the polariscope must have given us more positive evidence of such orientation than we have yet obtained. In the absence of such evidence the orientation-hypothesis possesses a metaphysical rather than a scientific character. We may juggle with the ultimate particles as we please and we may assume that they do all that is required to attain a certain result: unquestionably we shall succeed in interpreting all the phenomena of polarity in this way, but the value of our interpretations is chiefly academic, and scientific proof or disproof is impossible.

Summary.

1. The pieces of the actinian, *Harenactis attenuata*, which form "rings" by the union of oral and aboral ends about the whole circumference, after more or less complete removal of mesenteries and mesenterial muscles, may produce new discs with radially arranged new mesenteries and tentacles and mouth-opening and esophagus. Thus far the usual number of tentacles, twenty-four, has not been attained in any case, the largest number being sixteen. Such discs with from three to eight tentacles are of frequent occurrence, but the formation of mouth and esophagus has been observed only once. After the formation of the discs they may be gradually elevated from the surface of the rings by the development of a cylindrical column beneath them.

In addition to the well-developed discs, radially as well as bilaterally symmetrical and asymmetrical tentacle groups may arise along the line of union on either side, or the tissue of both sides may take part in the formation of a single group.

2. These further experiments with rings extend and confirm the earlier work. The new outgrowths on the rings represent a more or less close approach to new individuals and involve the establishment of new polarities and symmetries. They are to be regarded as a form of reproduction related to the formation of adventitious structures in plants. The localization of the outgrowths, as well as their polarity and symmetry, have no relation to the original polarity and symmetry, but are due to local conditions.

The results of these experiments can be more readily interpreted in accordance with the hypothesis that polarity and symmetry are essentially molar localizations or gradation of processes along an axis or axes, than with that which regards polarity and symmetry as the effect of a summation of the individual polarities and symmetries of protoplasmic particles or molecules which are definitely oriented with respect to an axis or axes.

Hull Zoölogical Laboratory,
University of Chicago,
November, 1910.

ON THE INTERCHANGE OF THE LIMBS OF THE CHICK BY TRANSPLANTATION.

FLORENCE PEEBLES, PH.D.

The experiments of Lillie ('04) and of Shorey ('09) have demonstrated that the injury of certain parts, and the removal of organs in the early stages of the embryonic development of the chick are rarely followed by regeneration of the lost part. Lillie found some evidence of the regeneration of the notochord, but a new wing did not develop after removal of the bud. From these experiments he drew the conclusion that the embryo of the chick possesses little more power of regeneration than the adult. Shorey also removed the wing buds of embryos of three to six days' incubation, and although the region healed and development of other parts proceeded normally, the wing buds did not show any sign of regenerative activity.

In the winter of 1908 I undertook a series of experiments, first to find out if the limb buds after removal could be grafted on again, and if so whether a leg bud grafted on the proximal part of the wing would develop into a wing or a leg, and vice versa, if a wing bud when grafted on the proximal part of the leg would develop into a leg or a wing.

Experiments of this kind on the chick are necessarily attended with many difficulties, so that even under the most favorable conditions, the percentage of successful operations is exceedingly small. My results, therefore, are largely negative, but it may be of interest to note what has been accomplished in the hope that more perfect methods will enable some one to obtain more satisfactory results.

Two general methods were followed. In the first series of experiments the eggs were left in the shell. A window was made above the embryo and after the operation it was sealed by the method which I ('98) used in experiments on the primitive streak. This method has since been used by other investigators with success. In the later experiments the eggs were removed from the shell at the end of the forty-eighth hour of incubation, and during the further development they were kept in porcelain cups in a moist chamber. The latter method was found more practicable, as the first appearance and the subsequent growth of the buds could be observed, and also the changes in the embryo after the operation could be watched from time to time.

The limb buds are not large enough for removal until the beginning of the fourth day, and allowing for the delay in development caused by the operation, at least four additional days of incubation are needed before the wings and legs show marked characteristics. At the end of the fourth day the embryo lies on its left side; for this reason the operations were made on the right side. The buds were removed by means of a curved knife made by heating and bending a no. 12 cambric needle into a hook and sharpening it at the curve. The hook was inserted under the bud and drawn up quickly, thus removing the tip with the least possible disturbance to the surrounding parts. The buds were then carried on the needle to the position desired, and held in place for a few minutes until they adhered. The greatest difficulty was met with in the effort to keep the grafts together. Some were fastened by fine glass threads, but this was ot found satisfactory as the insertion of the thread tore the tissues. In many of the embryos the grafts came apart, and the buds floated in the albumen or sank in the yolk. Another dif-

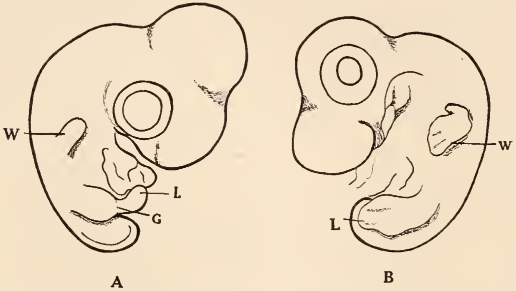


FIG. 1. Two views of a chick on the seventh day. *A*, the right side showing the stump of the wing *W* and the leg *L* with the line of the graft *G*. *B*, the normal side.

ficulty arose through excessive bleeding after the operation. This usually resulted in the death of the embryo.

A brief description of the behavior of one or two of the embryos will suffice to show the general results.

A. The leg and wing buds of an embryo, incubated two days in the shell and then two days in a porcelain cup, were removed. The leg bud was grafted on the proximal part of the wing, and the wing bud on the proximal part of the leg. The embryo was then placed in a moist chamber in the incubator where it was left for three days. On removal it was found that the graft in the wing region had separated, and that the bud on the proximal end of the leg was only partly attached (Fig. 1, *A*). The left side appeared as shown in Fig. 1, *B*. There was no regeneration in the wing region, and no apparent development in the leg region. Sections of this embryo showed the normal limbs to be composed of a homogeneous mass of mesoderm surrounded by ectoderm. The limbs on the right side were similar in structure. None of them showed distinguishing characteristics.

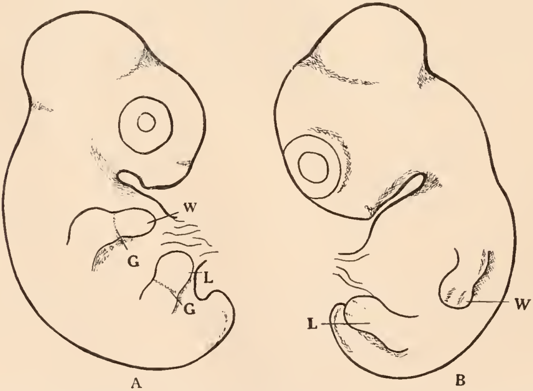


FIG. 2. Two views of a chick on the eighth day. *A*, the right side showing grafts; *B* the normal side.

B. The same experiment was performed on this embryo as on the one just described. In this case the chick lived four days after the grafts were made. Both unions were complete, as shown in Fig. 2, *A*, *G*. In Fig. 2, *B*, the left side is indicated. Sections of this chick show concentrated areas where the skeleton is beginning to develop. These areas are indicated in Fig. 3, *A*, *B*, *C*, and *D* by dots. A section of the normal side of the embryo is given in Fig. 3, *D*.

I have not succeeded in keeping any embryos alive beyond the ninth day of incubation, but a normal embryo of this age possesses wings and legs which are readily distinguishable. My results show that the operation greatly retards the development. If the embryos could be kept alive a few days longer it would, no doubt, be possible to determine positively whether or not the grafted tips become a part of the limb to which they are attached irrespective of their former position. The results of my experiments indicate that they do. The failure to keep the embryos alive is probably due to disturbance of the development of the allantois.

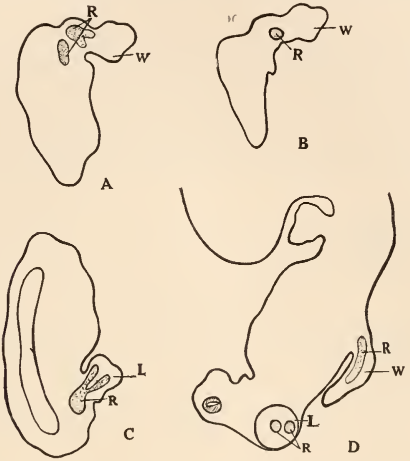


FIG. 3, *A* and *B*. Sections of the grafted wing *W* showing rudiments of the skeleton *R*; *C*, section of the leg *L* with the rudiments of the skeleton *R*; *D*, section of the left side. The leg *L* appears in cross section, the wing *W* in longitudinal section.

In regard to regeneration the results obtained from the removal of the leg and wing buds fully confirm those of Lillie and Shorey. In no case was the slightest sign of regeneration observed.

SUMMARY.

1. It is possible for chick embryos to develop in porcelain cups in a moist chamber at the proper temperature, up to the ninth day, although the development is delayed.

2. The leg bud when removed may be grafted on the proximal part of the wing and the wing bud may be grafted on the proximal portion of the leg without permanently injuring the embryo.

3. The results indicate that when the tip of a young bud is grafted on the proximal portion of another limb it becomes a part of the appendage to which it is attached instead of retaining the character of the part it is destined to become.

4. No regeneration of the limbs takes place after the removal of the buds.

Bryn Mawr College,
Bryn Mawr, Pa.,
November 12, 1910.

LITERATURE.

Lillie, F. R.

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Peebles, F.

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THE RELATION BETWEEN CHROMOSOME-NUMBER AND SPECIES IN NOTONECTA.[1]

ETHEL NICHOLSON BROWNE.

After working for several years on the spermatogenesis of *Notonecta*, some very interesting facts have recently come to light which I wish to present in a preliminary paper. The material, collected at Woods Hole, consists of three species, *Notonecta undulata* Say, *N. insulata* Kirby and *N. irrorata* Uhler. The character of the chromosome groups is quite distinctive for each species, *N. insulata* representing a transition stage between *N. undulata* with a larger number of chromosomes and *N. irrorata* with a smaller number.

In all three species there is present a pair of idiochromosomes, which divide separately in the first spermatocyte-division, as described by Wilson[2] for *Euchistus*, *Cœnus*, etc. Accordingly, there is one more than the reduced or haploid number in the first division. Owing to the fact that the conjugation of the idiochromosome-pair is often still further delayed in *Notonecta*, the two unequal mates frequently lie side by side on different spindle fibers in the second division (Figs. 7, 9, 16, 18, 33, 35). They subsequently pass to their respective poles without ever having conjugated. As a result of the second division, two classes of cells are produced, one containing the small idiochromosome and the other the large one (Figs. 10, 19, 36). Accordingly, the spermatozoa are of two kinds which, from analogy with other insects, are the male-producing and the female-producing spermatozoa respectively.

In *N. undulata* there are 14 chromosomes in the first spermatocyte-division, consisting of a ring of 12 chromosomes with two small ones in the center (Figs. 1–3). These small ones frequently give the appearance of being on the same spindle fiber (Fig. 4). There are 13 chromosomes in the second division, a ring of 12 chromosomes surrounding the idiochromosome-pair in the center (Figs. 5–10). The spermatogonial number is 26, including two pairs of small chromosomes, the same ones, evidently, which lie in the center in the first division (Fig. 11).

---

[1] I wish to express my thanks to Professor E. B. Wilson for his helpful suggestions and criticism during the course of the work, to the director of the Marine Biological Laboratory and the Wistar Institute of Anatomy for the facilities offered me at Woods Hole, and to Mr. E. P. Van Duzee for identifying the species.

[2] Wilson, E. B., 1905, "Studies on Chromosomes," I., *Journ. Exp. Zoöl.*, II., 3.

In *N. irrorata* there are but 13 chromosomes in the first division, a ring of 12 surrounding one small chromosome in the center (Figs. 12–14). In the second division there are 12 chromosomes including the idiochromosome-pair in the center (Figs. 15–19). The spermatogonial number is 24, the smallest pair of chromosomes corresponding to the small chromosome within the ring in the first division (Fig. 20).

In *N. insulata* there are two types of first division groups, occurring in the same testis, in approximately equal numbers. One type has 14 chromosomes, a ring of 12 surrounding two small ones (Figs. 21–23); the other type has but 13 chromosomes, a ring of 12 surrounding only one small one (Figs. 24–26). The apparent discrepancy is accounted for by the fact that the second small chromosome which lies in the center in the 14-group *is frequently found in the 13-group attached to the largest chromosome.* Serial sections of four spindles as seen in side view are given in Figs. 27–30. In the first three series (Figs. 27, 28, 29), there is only one small chromosome in the center of a ring of 12 chromosomes; the second small one is attached to the largest chromosome (*Ma*). In the fourth series (Fig. 30), the two small chromosomes lie free in the center of a ring of 12. A polar view showing the compound character of the large chromosome in the 13-group is given in Fig. 31 (*Ma*). No case of such a compound chromosome has been found associated with the 14-group, and some fifty clear cases have been observed in the 13-group. In many cases, however, the compound nature of the large chromosome could not be determined in the 13-group, the two components having evidently fused beyond recognition. The fusion must take place in all cases before the second division, for there are always, so far as I have observed, 12 chromosomes in this division, including the idiochromosome-pair in the center of the group (Figs. 32–36). All trace of the original composition of the largest chromosome has been lost. It has a decided quadripartite appearance, as though each element into which it divides were composed of two equal parts. Its appearance suggests that of the large chromosome described by Wilson[1] in the second division of *Nezara*, except that in *Nezara* the two parts are somewhat unequal. Unfortunately, no satisfactory spermatogonial groups have been found, but the expectation would be either 26 or 24, in the latter case, two of them being compound in character.

Discussion.

The chief interest in *Notonecta* lies in the fact that there is a definite relation between the chromosome-number and the species, and that the change in number can be attributed to the behavior of a particular chromosome. In *N. undulata* this chromosome is present as a separate element, together with another small one, in the center of the spindle in the first division, and in the peripheral ring in the second division. In *N. irrorata*, this chromosome is lacking throughout both divisions, there being only one small one in the center of the spindle in the first division. *N. insulata* gives a transition stage between the two; the small chromosome is present in the first division, either free in the center as in *N. undulata*, or fused with the largest chromosome; and it is apparently absent in the second division as in *N. irrorata*. Every chromosome cannot be homologized individually in the three species, for the size relations are different, especially in the case of the idiochromosomes. The largest chromosome and one small one can be followed throughout in the three species. Likewise, we may safely compare, I think, the other small chromosome in *N. undulata* with the one of like size and position that sometimes occurs in *N. insulata* in the first division; and since we can follow the steps of its fusion with the large chromosome in *N. insulata*, we may, I think, reasonably attribute its absence in *N. irrorata* to its permanent fusion with the large chromosome. A schematic representation follows, only the chromosomes that are comparable being separated from the ordinary chromosomes or autosomes, which are designated *A*. The large and small idiochromosomes are represented by I and i respectively, the largest chromosome, or macrochromosome by M, the small chromosomes, or small autosomes by a.

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[1] Wilson, E. B., 1910, "Note on the Chromosomes of *Nezara*," *Science*, May 20, 1910.

	Products of the First Division.	Products of the Second Division.
N. undulata	$M+9A+I+i+a+a$ (14)	$M+9A+I+a+a$ (13) and $M+9A+i+a+a$ (13)
N. insulata	either $M+9A+I+i+a+a$ (14) or $Ma+9A+I+i+a$ (13)	$Ma+9A+I+a$ (12) and $Ma+9A+i+a$ (12)
N. irrorata	$Ma+9A+I+i+a$ (13)	$Ma+9A+I+a$ (12) and $Ma+9A+i+a$ (12)

N. insulata evidently represents a transition stage between *N. undulata* with a larger number of chromosomes and *N. irrorata* with a smaller number. It is not possible to say which is the primitive condition. We might assume that *N. undulata* is the original species, from which, by a process of progressive fusion, have arisen the conditions seen in *N. insulata* and *N. irrorata*. We might, however, assume that the reverse process has taken place; or, that *N. insulata* arose together with one of the other species from the third species.

The somatic characters give no clue as to which of these interpretations should be adopted. We could easily derive the wing coloring of *N. insulata* from that of *N. undulata* by substituting brown pigment for white, and the wing coloring of *N. irrorata* from that of *N. insulata* by substituting black pigment for brown, but leaving some of the brown as mottling. But *N. irrorata* is intermediate in size between the small species *N. undulata* and the large species *N. insulata*. Of course the most striking somatic differences are not necessarily those correlated with the fusion or separation of the two chromosomes; these differences may be correlated with other chromosomes which, as I have stated, differ in size in the three species.

The fact that a transition stage has been found in one species of *Notonecta* between two other species of the same genus in respect to a fusion and separation of two chromosomes lends support to the views advanced regarding the origin of two or more chromosomes from a single chromosome where the transition stages do not exist. Payne[1] has concluded that the multiple X-element in the Reduviidæ has arisen from a single X-element of an ordinary idiochromosome-pair by a separation into two or more parts. Wilson[2] has accounted for the double accessory in *Syromastes* by assuming that it has arisen from a single chromosome that has split into two parts. These hypotheses are sustained by the evidence of *Notonecta*, two species of which show permanent fusion and separation, and the third species the intermediate condition.

Besides the interesting correlation of the change in chromosome-number and change in species in *Notonecta*, the condition of temporary fusion and separation of two chromosomes in one species, *N. insulata*, is of considerable interest, especially in comparison with the facts found in other insects. Sinéty[3] has found in the Phasmidæ and McClung[4] in other orthoptera that the accessory is sometimes attached to another chromosome; in *Mermeria*, McClung found this compound chromosome further associated with another chromosome. Wilson[5] describes in *Metapodius* a coupling of the supernumeraries with the idiochromosomes during part of the maturation-process. Wallace[6] has found in the spider that the accessory is made up of two components, sometimes united, sometimes separate. Similarly, in *Syromastes*. Wilson[7] has found that the accessory consists of two unequal elements which are closely united in the maturation-divisions. In the Phylloxerans, Morgan[8] describes two accessories, sometimes separate, sometimes united. It is to be noted, however, that in all these cases, the temporary separation or union is connected with the sex-chromosomes, whereas in *N. insulata* it concerns two ordinary chromosomes or autosomes.

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[1] Payne, F., 1909, "Some New Types of Chromosome Distribution and Their Relation to Sex," BIOL. BULL., XVI., 3, 4.

[2] Wilson, E. B., 1909, "The Female Chromosome Groups of *Syromastes* and *Pyrrochoris*," BIOL. BULL., XVI., 4.

[3] Sinéty, R. de, 1901, "Recherches sur la Biologie et l'Anatomie des Phasmes," *La Cellule*, XIX.

[4] McClung, C. E., 1905, "The Chromosome Complex of Orthopteran Spermatocytes," BIOL. BULL., IX., 5.

[5] Wilson, E. B., 1909, "Studies on Chromosomes," V., *Journ. Exp. Zoöl.*, VI., 2.

[6] Wallace, L. B., 1909, "The Spermatogenesis of *Agalena nævia*," BIOL. BULL., XVII., 2.

[7] Wilson, E. B., 1909, "Studies on Chromosomes," IV., *Journ. Exp. Zoöl.*, VI., 1.

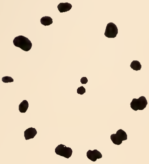
[8] Morgan, T. H., 1909, "A Biological and Cytological Study of Sex Determination in Phylloxerans and Aphids," *Journ. Exp. Zoöl.*, VII., 2.

MARINE BIOLOGICAL LABORATORY,
WOODS HOLE, MASS.,
September, 1910.

Explanation of Plate I.

Notonecta undulata. Figs. 1, 2, metaphase of first division showing 14 chromosomes, two small ones in the center. Figs. 3, 4, initial anaphase of first division, side view, two small pairs in the center. Fig. 5, metaphase of second division showing 13 chromosomes, large idiochromosome in the center. Figs. 6, 7, the same, but both idiochrosomes showing in the center. Fig. 8, initial anaphase of second division, side view, idiochromosome-pair in the center. Fig. 9, the same, but idiochromosomes on different fibers. Fig. 10, *A* and *B*, sister anaphase groups of second division, from the same spindle. Fig. 11, spermatogonial group showing 26 chromosomes.

All the figures were drawn with a camera, 1/12 oil immersion and a 12 compensation ocular, then reduced in the engraving to two thirds.



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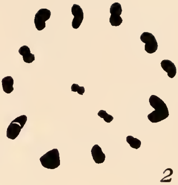

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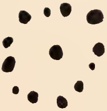

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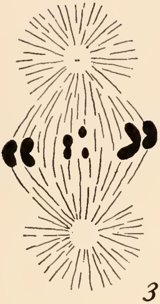

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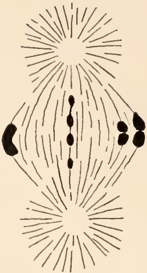

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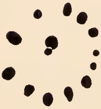

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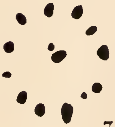

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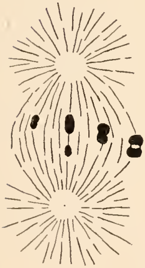

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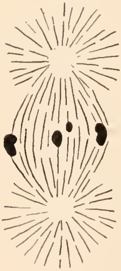

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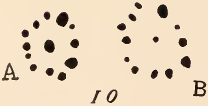

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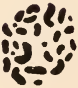

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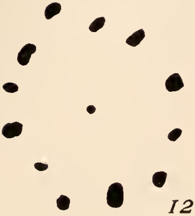
ETHEL N. BROWNE.

Explanation of Plate II.

Notonecta irrorata. Figs. 12, 13, metaphase of first division showing 13 chromosomes, one small one in the center. Fig. 14, initial anaphase of first division, side view, one small pair in the center. Fig. 15, metaphase of second division showing 12 chromosomes, large idiochromosome in the center. Fig. 16, the same, but both idiochromosomes showing in the center. Fig. 17, initial anaphase of second division, side view, idiochromosome-pair in the center. Fig. 18, the same, but idiochromosomes on different fibers. Fig. 19, *A* and *B*, sister anaphase groups of second division, from the same spindle. Fig. 20, spermatogonial group showing 24 chromosomes.



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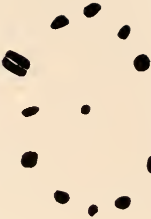

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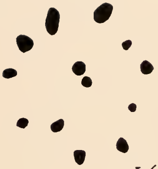

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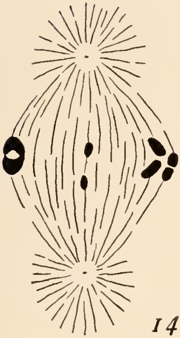

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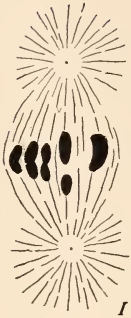

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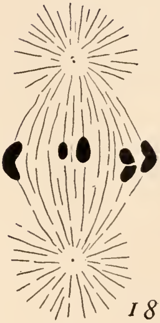

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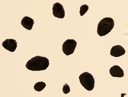

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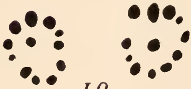

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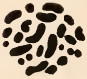
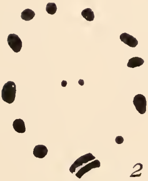


20

ETHEL N. BROWNE.

Explanation of Plate III.

Notonecta insulata. Fig. 21, metaphase of first division showing 14 chromosomes, two small ones in the center. Figs. 22, 23, anaphase of first division, side view, two small pairs in the center. Figs. 24, 25, metaphase of first division showing 13 chromosomes, one small one in the center. Fig. 26, initial anaphase of first division, side view, one small pair in the center.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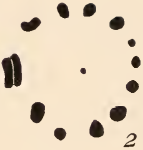
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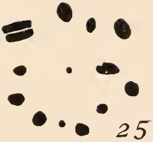
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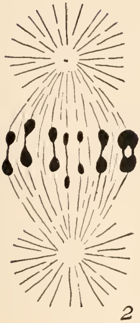
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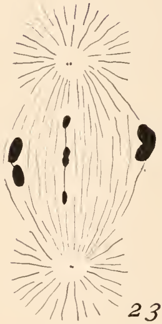
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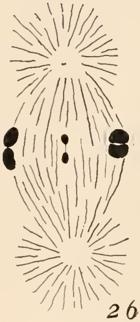
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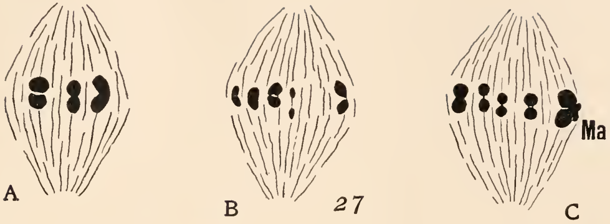
ETHEL N. BROWNE.

Explanation of Plate IV.

Nonecta insulata. Serial side views of four spindles of first division, as seen in adjacent sections. Figs. 27–29, *A*, *B*, *C*, three entire spindles each showing 13 chromosomes, one small pair free in the center in *B*, and a compound chromosome in *C* (*Ma*) consisting of the large and the other small chromosome united. Fig. 30, *A*, *B*, *C*, entire spindle showing 14 chromosomes, two small pairs free in the center in *B*; the two elements of the compound chromosome are separate as *M* and *a* in *C* and *B*.

Some of the chromosomes have been displaced on the spindles so as to lie side by side instead of one above another, for the sake of clearness.



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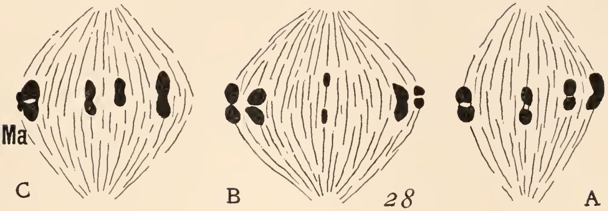

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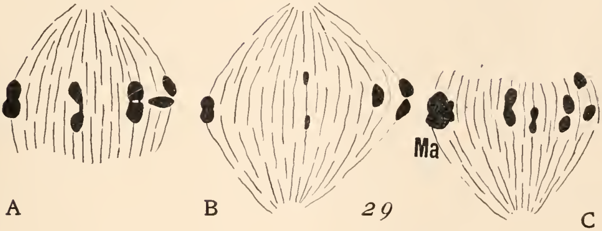

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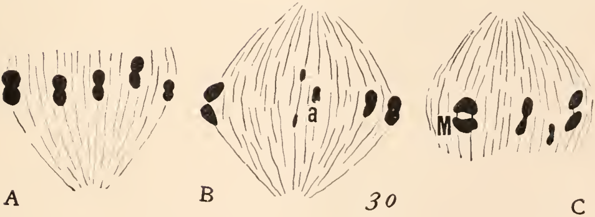

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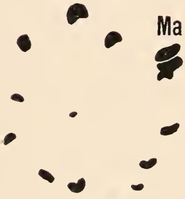
ETHEL N. BROWNE.

EXPLANATION OF PLATE V.

Notonecta insulata. Fig. 31, metaphase of first division showing 13 chromosomes, one small one in the center, the other small one attached to the large one forming the compound chromosome *Ma.* Figs. 32, 33, metaphase of second division, showing 12 chromosomes, both idiochromosomes showing in the center, Fig. 34, initial anaphase of second division, side view, idiochromosome pair in the center. Fig. 35, the same, but idiochromosomes on different fibers. Fig. 36, *A* and *B*, sister anaphase groups of second division, from the same spindle.



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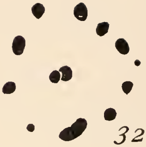

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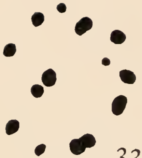

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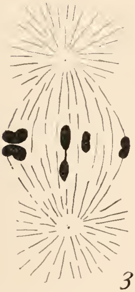

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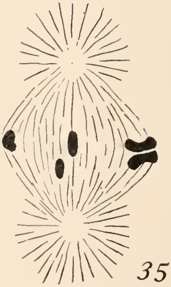

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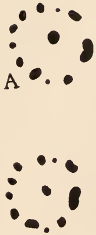

36

ETHEL N. BROWNE.

SOME RESULTS OF CASTRATION IN DUCKS.[1]

H. D. GOODALE.

Introduction.—The present paper is largely in the nature of a first report and will be followed by others as the experiments warrant. In addition to a brief review of some other studies on castration, I have considered, also very briefly, some problems relating to sexual dimorphism, sex-limited inheritance and the determination of sex.

It is a matter of rather common knowledge that females of birds and mammals, which have become sterile from any cause, may assume the plumage and other secondary sexual characters of the male. On the other hand, the male when castrated, often does not develop his usual trappings. This has often been interpreted as a development of the female characters. It has, however, been pointed out that castration of the young male causes rather a retention of his own youthful characters.

Castration, besides its effect on the secondary sexual characters, may lead to certain specific changes in the organism, not correlated with secondary sexual characters. Thus the capon grows larger than the cock and is more sluggish. Tandler and Grosz found in man, among other things, that there was a tendency for fat to develop in certain regions, and either a failure, or poor development of body, axial, and pubic hairs.

The present paper deals almost wholly with the effect of castration on the secondary sexual characters.

The breed known as Rouens was selected for this work, largely because they are strongly sexually dimorphic in plumage.[2]

The Rouens are probably derived from the mallard, *Anas boschas*. Coues says, "nearly everywhere domesticated, being the well known original of the barnyard duck." In some respects the Rouens differ from the mallard. The latter are much smaller.

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[1] For the suggestion of studying the effects of castration in ducks, I am indebted to Professor T. H. Morgan.

[2] The individuals used in these experiments were reared from eggs secured from the White Birch Poultry Farm, Bridgewater, Mass.

The tail of the male has some white, while in summer plumage he resembles the *female Rouen*. (For description of latter see below.) The female mallard, though of the same general color, is of much lighter tone throughout than the Rouen female, which might easily pass as a melanistic variety of the former. For practical purposes we are dealing with a wild species.

In addition to a general description, I have given a somewhat detailed description of the feathers of the various sections of the birds. There is, however, so much variation in the patterns of the feathers of the female and the male in his summer plumage, that these descriptions and figures illustrate only a few types. This variation exists, not only between different birds, but even in a single section of one individual. Though two feathers are rarely exactly alike, the majority of feathers in a single section have a general resemblance. On the other hand the feathers of each section of the male's breeding plumage are very constant in type.

General Descriptions.

Male in Breeding Plumage (Fig. 1).—Bill: greenish yellow. Head and upper part of neck: rich metallic green. Then comes a narrow white ring often not quite complete dorsally. Ventral side of remainder of neck and a large part of breast: rich claret. Remainder of ventral surface: iron gray, becoming lighter toward the anus. Posterior to the last it becomes darker. Back: very dark gray in neck region, becoming still darker in the middle and greenish black on the rump. The two median tail feathers are strongly curved antero-dorsally. They have the same color as the rump. The next two are often curved, but may be duller in color. These four form the so-called sex feathers. The rest of the tail and the upper surface of wing is brownish black. The latter has a speculum of iridescent purple. Under surface of wing: white. The drake's voice is a soft qua, either rapidly repeated, with a slight pause after every other note, or else a somewhat louder, single note, much prolonged. In some varieties the voice and sex feathers are the only conspicuous secondary sexual characters.

Female (Fig. 2).—Throughout a mixture of buff and dark brown. Bill: brownish black, usually mottled with yellow. Occu-

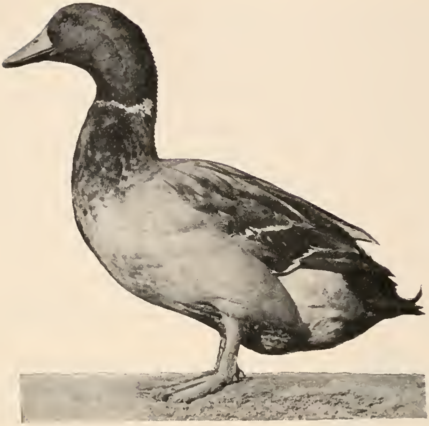

FIG. 1. Male in breeding plumage.

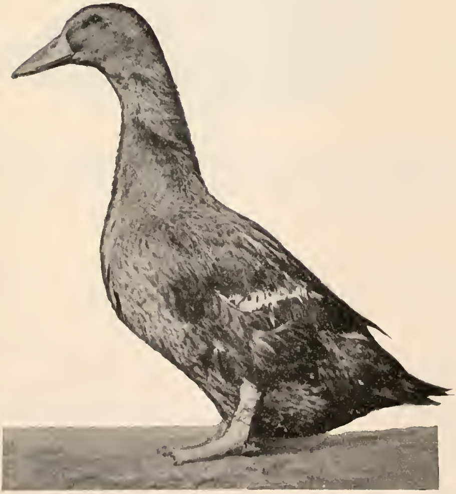

FIG. 2. Normal female.

pying the dorsal side of the head and upper neck is a broad dark stripe, tinged with green and inconspicuously striped with buff. The sides of the head are buff with small dark flecks. Passing across the side of the head from the nostril through the eye to the dorsal surface and from the angle of the jaw to the eye, are two dark bands. Sides of upper neck: mixed buff and dull black. Throat: buff. Dorsal surface of body often much darker than ventral, and usually less conspicuously striped. Speculum and under surface of wing as in the male. Sex feathers absent. Voice a loud quack. At times it is modified into a softer, rapidly repeated quac.

Summer Plumage.—At the close of the breeding season, which comes early in July, both sexes molt. The new coat of the male, when complete, is different from the old (Fig. 3). The molt, however, takes place piece-meal, certain sections being nearly completed before others begin. Consequently there is only a very brief period at the end of the summer when the new coat exists as a whole. Then the transition begins again to the usual coat which is completed early in October. In the modified coat the male is said to be in "summer plumage," or in a "state of eclipse."

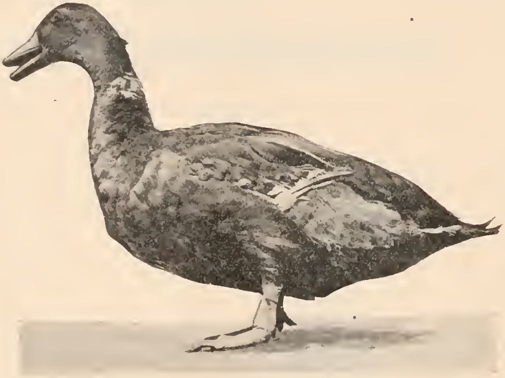

FIG. 3. Male in summer plumage.

Head: similar to that of female. Breast: mottled dull black and reddish buff. Keel: dull slate with buffish streaks. Remainder not conspicuously modified. Sex feathers absent.

The female, so far as I have observed, undergoes no radical change in coat color, though in one case, at least, the patterns of the feathers were considerably different in August from those of October. Compare Fig. 4, *E* with *F*; Fig. 6, *D* with *F*.

As a result of the molt in early summer, both sexes of the adult and the young are quite similar for a time. Its meaning is uncertain. Newton ascribes the assumption of the summer plumage by the drake to the loss of the power of flight temporarily, since the remiges all fall out together. But in other cases where a similar double molt occurs, the male retains the modified plumage for several months; nor does he become incapable of flight. As will be shown later, the presence of the active testis is necessary for the drake to assume this plumage. Conditions in the mallard, as far as I can find out, appear to be somewhat different from the Rouen. The summer plumage seems to persist longer. The one male in the American Museum in a transition stage, had a brown edging to the feathers of regions, which, in the male Rouen, have no such edging.

Young.—When first hatched the young are brownish black with a few yellowish stripes or spots. While they are acquiring their first coat of feathers both sexes resemble the female in general appearance. This summer, however, I found certain characters of the plumage by which the sexes could be distinguished at a very early age (Fig. 8, *D*, *E*, from birds about 7 weeks old). The young "peep" for several weeks after hatching. The voice of the young female is modified into that of the adult a few weeks before that of the young drake. The latter is about four months old when he takes on the adult plumage. At this time the testes are still very small.

Description of Feathers.[1]—Male in breeding plumage.—Head and upper neck: distal half, metallic green; proximal half, dull black with narrow white base; on the ventral side of head, this base may be more extensive. Neck ring: entirely white. The feathers of regions immediately adjoining the neck ring may be many kinds of mixtures of white, red and black. Ventral surface.

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[1] The words distal and proximal refer to the feathers.

—Lower throat: distal half, deep claret; proximal half slate, sometimes stippled or spotted with buff. Breast: distal half claret; remainder slate, becoming white at base, usually stippled with buff (Fig. 4, *A*); sometimes numerous transverse vermiculations are present (Fig. 4, *B*); less frequently, still other slight modifications may occur. Keel (Fig. 5, *A*, *B*) and laterals:[1] light gray becoming darker proximally; distal half traversed by numerous transverse slate colored vermiculations;[2] near the breast region the tip of the feather may be shaded with faint claret (Fig. 5, *B*). Posterior to vent: (Fig. 8, *I*) similar to preceding, but the slate vermiculations are much broader and often become confluent, forming patches. Sides posterior to folded wing: (Fig. 8, *J*) similar to keel but much lighter; vermiculations often broken; proximally: slate at base, but more distally often changes abruptly to white. Under tail converts: black becoming lighter toward the base; exposed surface with metallic sheen. Dorsal surface.—Lower neck near body: slate, becoming somewhat lighter toward the base; light gray vermiculations distally (Fig. 6, *A*). Passing back along the back the gray vermiculations tend to disappear except at the edge of the feathers. The slate also gradually gives place to black which as it nears the rump becomes metallic blue or green on its exposed surface. Rump (Fig. 7, *A*), upper tail coverts and sex feathers: black with metallic green or blue on the exposed surface; vermiculations rarely occur. Main tail, primaries and primary coverts: dull brownish black. Secondaries: a narrow white band across the tip; remainder, outer surface, dorsal side of vane: dull brownish black; ventral side of vane: next to white tip a narrow velvety black band; the rest, except for a narrow dull band near the base is brilliant changeable purple, blue or green; inner surface, dull brownish black. The innermost secondaries: slate, often with velvety black. Secondary coverts: velvety black band across the tip, then a narrow white band, remainder brownish black. Remainder of upper surface of wing: brownish black. The feathers at the anterior edge have gray vermiculations which disappear posteriorly. Scapulars: various modifications of the uniform slate and gray vermiculated types. There is also a peculiar tuft of small feathers just anterior to the junction of the wing and body. Each feather (Fig. 8, *A*) has a narrow band of slate and light gray vermiculations across the tip, then a curved band of light claret, the remainder being a mixture of slate and gray.

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[1] On the sides of the body just beneath the edge of the folded wing is an elongated patch of very large feathers, which I have thus designated.

[2] I have arbitrarily called the *narrowest* bands vermiculations.

Male in Summer Plumage.—Top of head: almost identical with normal type, but much less brilliant. Light line above eye: distal half mostly buff with small irregular blotches of black; base, dull black; sometimes the entire feather is nearly black. Beneath the eye: dull black; distal half with buff edges. Throat: reddish buff with irregular dull black marks; basal half slate. Upper breast: (Fig. 4, *C*) bands of reddish buff or claret and dull black; though irregular they are more or less transverse; basal half: slate. Regular patterns may occur (Fig. 4, *D*). Lower breast: buff in place of reddish buff; otherwise as preceding; sometimes feathers like those of one of the female types are found (Fig. 5, *D*). Anterior keel: (Fig. 5, *C*) mostly slate; margin of varying width vermiculated with gray; tip usually of rufus shade. Posterior keel: dull slate, vermiculated margins reduced or absent. Posterior to vent: dull black with vermiculated margins. Under tail coverts: dull black with narrow buff margin on exposed part, sometimes vermiculated. Sides of body posterior to wing: (Fig. 8, *H*) slate with narrow gray vermiculations. Dorsal surface of body: modifications often slight throughout; the feathers at union of neck with body (Fig. 6, *C*) lose most of their vermiculations and may have buff edges. Scapulars: dull slate. Laterals somewhat modified, often with a distinct tendency for the formation of transverse reddish buff and black bands across the tip, similar to those of the breast; vermiculations reduced or absent.

Female.—Top of head: distal half, dull greenish black with buff margins; basal half slate. Line above eye: reddish buff with median dark stripe; basal half slate. Throat: reddish buff with gray base. Upper neck at junction with next section: buff with irregular longitudinal dark stripes; base slate. Breast: buff, sometimes reddish, with dark patches, arranged in numerous patterns (Fig. 4, *E–H*). Keel: (Fig. 5, *E*, *F*) similar to preceding but usually duller and if anything less regular in pattern. Near the anus the feathers are apt to show a more regular pattern similar to Fig. 4, *E*. Dorsal surface: anteriorly (Fig. 6, *D*, *E*), rather brighter colors than elsewhere and often a distinctly more regular pattern. Fig. 6, *D*, closely approximates the fancier's ideal. Posteriorly the pattern becomes variable again and colors duller (Fig. 7, *C*, *E*). In some females the entire dorsal surface is almost black, as the buff markings become faint (Fig. 7, *E*). Main tail: brownish black, with narrow buff margins. Junction of wing with body: (Fig. 8, *C*) light rufous with irregular black spots. Scapulars: various modifications of "ideal" type. Upper surface of wing not including remiges: dull black with buff margins. Remainder of wing like males except that inner secondaries have some tendency toward developing white bands.

Young.—Unfortunately I have only a few notes on the feathers of the young. In one young female, the breast (Fig. 8, *E*) and keel feathers were dull brownish black with a buff margin, which is very narrow at the apex. The rump feathers were black with a narrow subapical buff band bounded by a margin of black.

In two young males the breast feathers were dull black, the distal half margined with buff and having two narrow transverse subapical buff bands (Fig. 8, *D*). The keel feathers were like those of the young female. The rump feathers were black like those of the adult male.

The general appearance of the young female, even before she gets a full coat of feathers, is like that of the adult. The young male, too, resembles the female, until one learns the characters which distinguish him from his sisters.

The Relation between the Summer Plumage of the Male and the Female's Plumage.

These descriptions show that the male in summer plumage merely mimics the female. He does not even become entirely like her. In certain sections, as pointed out, there are no modifications toward the female type. In others, *i. e.*, the head, breast and keel region, the feathers of the male become quite like those of the female. Individual feathers may be indistinguishable from female feathers. But the male in addition has types of feathers which the female lacks. Thus, the feathers of the top of the head have no buff edges. Those of the posterior keel region may have gray vermiculations on the margins. Those of the throat and upper neck regions, however, appear to be nearly identical with female feathers of the same sections. The breast feathers present some complications. Certain types of these are common to both sexes, but the male has certain forms not possessed by the female. The differences, though elusive, may perhaps be stated as follows: The apical part of the male's breast feather is claret; that of the female is buff. There is a tendency in the male for the red or buff bands to increase from two to three and to become transverse. In the female the tendency is for the bands to become reduced in number and to become longitudinal. Only a statistical study could determine just the exact relations between the two sexes.

This discussion, I think, makes it clear that the modifications in the plumage of the male are, on the whole, of a type peculiar to his sex. It can hardly be maintained that this is an example of the assumption by the male of the female's plumage, especially as I shall show later, the presence of the testis is necessary for its appearance. The power to change his plumage is, indeed, a sort of physiological secondary sexual character.

EXPERIMENTS.

Thus far I have castrated 7 males and 5 females.[1] Three males and two females lived for considerable periods. No. 470♂ and 4♀ were castrated in early spring, 1909, when a little less than a year old. The right testis of No. 19♂ was removed at this same time, but the left was not removed till August 8. The left testis of No. 1♂ (hatched spring 1908) was removed August 8, but owing to excessive bleeding, the right was well ligatured and allowed to remain. These two males were in summer plumage when operated on. No. 24♀, 12 weeks old, was castrated August 13. No. 1♂, No. 24♀ and No. 4♀ are still alive.

Results.—Males: No. 470 did not take on the summer plumage in 1909. He was unfortunately killed by dogs in spring of 1910. No. 1 molted in August, 1910, but did not take on the summer plumage except for a few feathers of head, throat and breast. No. 19 with one testis and this injured assumed the summer plumage in 1909. He did not long survive the second operation.

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[1] Not including several others which died in the early part of the work.

Evidently the presence of the active (?) testis is necessary for the male to assume the summer plumage. The presence of the ligatured testis of No. 1 indicates that ligaturing is as effective as removal. I do not know the present condition of the testis of this male. There was no assumption of female characters by any of these males.

Females: No. 4 and No. 24 were the only females that lived more than a few days after the operation. October 20, 1909, they showed no marked modifications, though a small sample of No. 24's breast feathers, saved at that time, are now found to be quite like those of the male in summer plumage. Those of No. 4 were of the usual female type. Through the winter and spring, these two, with several other females were confined in a rather dark pen. During this time, only cursory observations were made, for it was believed that the experiments had failed. July 4, 1910, this flock, for the first time in several months, was

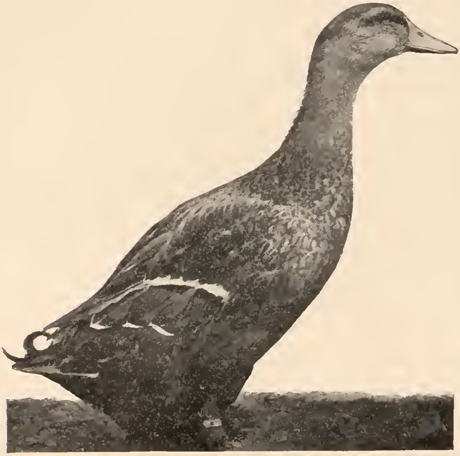

Fig. 9. Female 4, castrated.

given their liberty out of doors. This procedure at once revealed that two individuals carried sex feathers. As no males were with the flock, they were immediately examined carefully. Their band numbers, of course, provided the necessary identification. No. 4 (Fig. 9) in addition to the sex feathers, had breast feathers similar to those of the male's breast in summer plumage. In a few feathers, gray vermiculations were present. Such other modification as had taken place (*e. g.*, very little buff in dorsal regions) was not beyond the range of variation in normal females. No. 24, Fig. 10, was rather more strongly modified. She had a very narrow white neck ring. The feathers of the breast were distinctly of the male type (Fig. 4, *I*, *J*). Those of the dorsal side of the lower neck region were of a type occasionally occurring in the same region in males. In other parts of the body there was a distinct tendency for the buff bands to become transverse. Compare Fig. 6, *G*, *H*. Feathers, more or less vermiculated, were quite common. A few found in the anal region, were indistinguishable from similar male feathers. Compare Fig. 8, *G* with *J*.

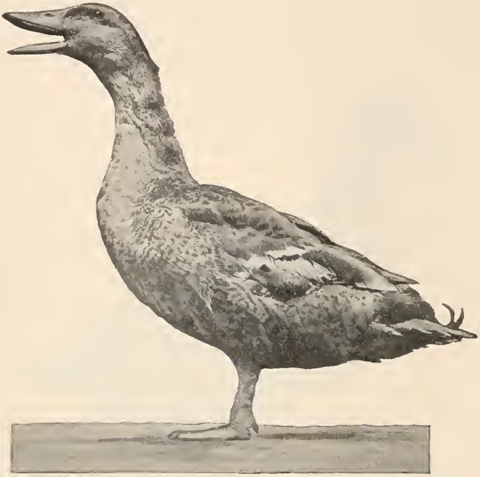

FIG. 10. Female 24, castrated. Photographed August.

The next molt of these birds, begun in September, is now (November) well advanced. On the whole No. 4 has made little advance toward the male type. There is much more buff in the dorsal regions than previously, but as stated above, the diminution of buff as noted in the summer was not beyond the range of variation in normal females. On the other hand, a distinct neck ring is now present where earlier there was only a trace.

No. 24 (Fig. 11), on the contrary, has made a distinct advance toward the male type. Compare Fig. 10 with Fig. 11. A comparison of Fig. 11 with Fig. 3 shows how closely she resembles the male in summer plumage, even though there are still many unshed feathers of last summer's coat.

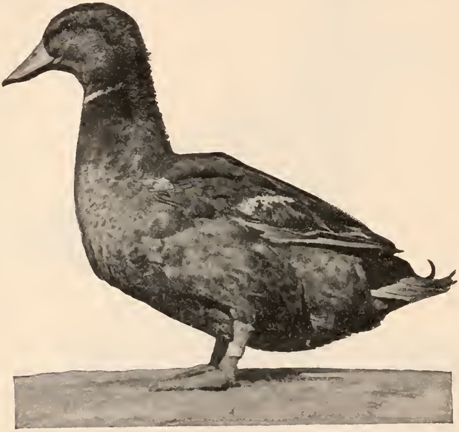

FIG. 11. Female 24, castrated. Photographed November.

Description.—Head mostly that of female type but in addition numerous brilliant green feathers just like those of the male have developed, though at present they are confined to the dorsal surface. Neck ring better developed than previously. The feathers of the dorsal surface are very similar to those of the male (Fig. 6, *I*, Fig. 7, *G*, *H*), though on the back towards the neck, feathers like those shown in Fig. 6, *G*, *J*, are common. The new feathers of the keel are shown in Fig. 5, *H*, *I*. The upper one is the more common. Its transverse buff bands recall those of the young male. The new feathers around the vent and sides of body posterior to wings (Fig. 8, *G*, *F*) are largely of the male breeding plumage type, though those of the sides are somewhat darker, more like the feathers of the same place in summer plumage. The new laterals are of the male summer type. Conditions in the breast region are rather complex. The upper part as a whole, is a deep claret, gradually becoming lighter ventrally and shading into the keel region. There is no sharp boundary as in the male. A feather from the upper (anterior) region is shown in Fig. 4, *L*. Further down on the breast, types like that shown in Fig. 4, *K*, are very common. The types shown in Fig. 4, *B*, Fig. 5, *I*, and Fig. 6, *G*, also occur, the last being especially frequent at the sides.

This female evidently is still undergoing modifications and in due course of time may assume the complete plumage of the male. At present she has the following distinctively male characters. Brilliantly green feathers on head; white neck ring; much claret in breast and some feathers indistinguishable from the male's; numerous vermiculated feathers, often identical with those of the male; rump feathers like that of male; and sex feathers. I have not seen feathers like those shown in Fig. 5, *H*, *I*; Fig. 6, *G*, in either normal sex, though feathers suggesting the last have been seen in the male: The few feathers of purely female type remaining are in the head and upper surface of wing. The color of the bill has not changed. The voice, too, is still the female's, though it has a tendency to break.

All that can be said in regard to sexual behavior is that the castrated females seem quieter than the others. Fuller observations will be made when the breeding instincts of both sexes become more active.

While the difference in plumage between these females may be due to the difference in age when castrated, no discussion of this point will be attempted in the present paper.

Discussion.—The results of these experiments show that removal of the testes does not bring about the assumption of the female characters, but at most results in the loss of a male character. The loss of the power of taking on the summer plumage is similar to the loss of power by the castrated stag of renewing the antlers each year. Whether castration of the drake, very early in life, will prevent the assumption of the breeding plumage will be determined later.

Removal of the ovary, however, has an entirely different result. The female after a time may gradually lose her normal characters and assume those of the male. The results entirely confirm previous observations along this line. Darwin records a duck which in old age assumed the perfect winter and summer plumage of the drake. Korscheldt also records a similar case.

Similar changes occur in fowls. The capon is well known. He is larger, heavier, "softer" and more sluggish than the normal male. He is said never to crow. Comb and wattles are poorly developed, but the other secondary sexual characters may be fully developed. Shattock and Seligmann, however, appear to have found that this development takes place only when some testicular material remains behind. But the age of the bird when castrated may have an important bearing on this point. Stags when castrated very young develop only a very small spike, though the castrated adult retains large antlers. Likewise, a bull, castrated when mature (called a "stag"), differs little from the normal adult. The bull, castrated as a calf, on maturing differs in several respects from the normal adult. Castration of the cockerel, then, has two results. First, it may result in the failure of some of the secondary sexual characters to develop; second, it brings about certain other modifications not associated with secondary sexual characters. There is an interesting peculiarity of the capon. He is said to be capable of being trained to brood and care for chicks. It is doubtful, though, if this is the acquirement of a female sexual character, for he apparently does not become broody, but merely is easily trained to care for the chicks. Poulards are less well known, but may be expected to develop male characters. Waterton records such a case, not resulting, however, from surgical castration (cited by Darwin).

In mammals the general results seem to be quite similar. The castrated female is commonly supposed to assume male characters. The male on the other hand merely fails to develop his secondary sexual characters, which remain in a more or less youthful condition. In addition castration may have specific results, not associated with secondary sexual characters. Tandler and Grosz have recently made an extensive study of eunuchs which may be briefly and incompletely summarized as follows: The individual tends to retain youthful characters. Thus the larynx is like that of a boy; the voice high pitched and likely to break similarly to that of a boy at puberty; beard, body, axial and pubic hairs sparse or wanting. The epiphyses remain open for a long time, consequently the eunuch is usually very long limbed. Fat tends to develop in certain parts of the body. The skin is usually soft, poor in pigment, and of a peculiar yellowish color. Facial wrinkles of a peculiar type are also found. The effects of castration of male deer and cattle have been noted. Less is known of the doe, but horns sometimes develop, apparently as the result of abnormal ovaries (Rorig). Similarly in the Hardwick breed of sheep, which are horned in the male only, the horns fail to develop after castration (Shattock and Seligmann). In reindeer, however, where both sexes are horned, castration does not affect the development or renewal of the horns. Other vertebrates have been little studied. Nussbaum, however, finds that the nuptial organs of the male frog do not develop on castration.

Experimental castration in insects has been studied by Oudemans, Kellogg, Regen, Meisenheimer and Kopec. The last two have also transplanted the gonads from one sex to the other. They agree that no modifications of the secondary sexual characters occur. Wheeler, who has recently made an extensive review of the subject, including its physiological side, reaches the same conclusion.

In some other arthropods, however, very different results have been obtained. Giard, Smith and Potts have studied parasitic castration in various crustacea. Their results may be summarized thus: The secondary sexual characters of the female remain unaltered, but the male becomes more or less modified in the direction of the female. In extreme cases, after recovery, ova have been found in the testes, the individual having become hermaphroditic.

This brief review shows that castration varies in its influence in different groups. In a given species, castration remodels the secondary sexual characters of only one sex. The case of the stylopized Andrenidæ recorded by Perez, in which reciprocal modifications took place, seems in the light of present knowledge to be somewhat doubtful (Wheeler). At present, then, the effects of castration, so far as it affects the secondary sexual characters, agree with breeding experiments in indicating that one sex only is heterozygous for sex.

The view that the male in birds owes his secondary sexual characters to the addition of something to the female type, is not supported by these experiments on ducks, for it is quite clear that the female owes her color to the ovaries or something associated with them, which previous to castration, suppressed the male characters and which insured the development of her own type of plumage. The female's plumage is obviously a protective adaptation. From an evolutionary standpoint, it is quite as conceivable, that selection should operate to pick out the inconspicuously colored females, as that the greater vitality of the male, or a selection of the more brilliantly colored males by the females, should bring about an addition to the female's type of plumage.

For the present, I think it makes little difference whether we assume that the ovaries themselves or a modifier always transmitted with the ovaries, prevents the development of the male characters. The evidence of the possible presence of a modifier, however, renders possible a somewhat different suggestion regarding the mode of sex-limited inheritance than has previously been expressed.[1] It involves also a number of possibilities regarding the inheritance of sexual dimorphism of plumage.

The Brown Leghorns are highly sexually dimorphic in plumage. In cross breeding it is found that the female transmits this color to her male offspring only, though the male transmits it to all his offspring. Barring, likewise dimorphic though in less degree, is transmitted is the same way. But that this is a universal rule for poultry with sexually dimorphic plumage appears to be somewhat doubtful. The penciling of the Dark

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[1] Sex-limited inheritance appears to occur in ducks. A paper on this subject in preparation.

Brahma female seems to be transmitted to the female offspring only, without regard to whether the Brahma is mother or father (Davenport). But there are some possible complications resulting from the particular matings made, which make this point uncertain. Thus far, however, the study of sex limited inheritance including the invisible factor "D" described by Bateson for the Brown Leghorn and the castration experiments on ducks all point in the same direction.

Some of the possibilities in the mode of inheritance of sexually dimorphic plumage are as follows:

1. In the female we may assume that the plumage occurs as a typical Mendelian heterozygote of male color and female color,[1] the former being considered recessive. The male, then, must be a homozygous recessive. But such assumptions do not agree with the results of breeding experiments as mentioned above for they show that in the female, a color factor occurs in only half her gametes.

2. Among the various other ways of representing the mode of inheritance of sexually dimorphic plumage, the following seems the nearest approach to our present knowledge. Assume that the male is homozygous for sex, the female heterozygous; that the male color (or color factor) *mc* also is homozygous in the male, but in the female heterozygous, the other element of the pair being an invisible modifier, *M*, which always couples with femaleness; or, assume that the male color always couples with maleness. Then the male is $\underset{♂}{mc}\ \underset{♂}{mc}$, gametes all $\underset{♂}{mc}$; the female is $\underset{♂}{mc}\ \underset{♀}{M}$, gametes $\underset{♂}{mc}$, $\underset{♀}{M}$. The breeding experiments mentioned above afford the reasons for assuming that the male is homozygous for sex, and the female heterozygous, and for the assumed couplings. The castration experiments give the reason for assuming a male color and a modifier. The expression, male color, however, is used in a purely descriptive sense. I do not wish to be understood as giving it any special significance.

3. By the additional assumption of selective fertilization, the male can be shown to be heterozygous instead of the female,

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[1] *E. g.*, male Brown Leghorn color and female Brown Leghorn color as such.

thus: Male $\underset{\text{♂}}{mc}\ \underset{\text{♀}(x)}{mc}$, gametes $\underset{\text{♂}}{mc}$, $\underset{\text{♀}(x)}{mc}$; female $\underset{\text{♀}(x)}{mc}\ \underset{\text{♀}(x)}{M}$, gametes $\underset{\text{♀}(x)}{mc}$, $\underset{\text{♀}(x)}{M}$. The only matings to be regarded as possible are $\underset{\text{♀}(x)}{mc}$ by $\underset{\text{♀}(x)}{M}$ and $\underset{\text{♀}(x)}{mc}$ by $\underset{\text{♂}}{mc}$.

The x in parenthesis is given as an alternative mode of representing sex by the x-element. This mode of representation would bring the experimental results into line with Guyer's discovery of an accessory in fowls.

The modifier assumed can hardly be specific for every type of dimorphism, else in cross breeding the later generations of females should show various modifications of the original female type. Of this there is no evidence. The modifier may, of course, be femaleness itself, in which case a somewhat different mode of representation should be employed.

It is obvious that Guyer's discovery of an accessory in fowls is contradictory to the results of experimental study which indicate the correctness of the Bateson-Spillman theory of sex in fowls, unless selective fertilization is assumed. Even if this be done, the male color obviously cannot be transmitted by the idiochromosomes. There is, of course, the possibility that the x-element is not a sex determiner at all, but merely a further indication of sex dimorphism. On the other hand the discovery of cocks which in cross breeding transmit some character to their female offspring only, would disprove the Bateson-Spillman theory. Hagedoorn seems to have found such a case, but he does not seem to have noticed its implications. A reëxamination is desirable.

Before proceeding further, a brief account will be given of some modifications of sex.

Braem, while studying regeneration, removed the last 22 segments of 35 in a female *Ophryothrocha*. Later he found that the ova in the anterior sections were degenerating and spermatozoa developing. Hermaphrodites often occur in *Ophryotrocha*, but in these the male germ cells always develop first, exactly the reverse of the present case.

During the past summer, my attention was attracted to certain changes in sex in maize, which is monœcious. The maize plant consists of a single stalk, at the apex of which is the tassel bearing the anthers. The female reproductive organs, forming the "ears," arise from axils of the leaves about midway of the stalk. In some varieties secondary stalks called suckers may arise near the base of the plant. They are usually devoid of ears, but almost always produce a tassel.

Sometimes, however, kernels of corn develop on the tassels. These appear to be always associated with a smut fungus. These kernels were often very numerous on the tassels of suckers, occasionally a fair resemblance to an ear being formed.

Stamens are less easily found on the ear but they may occur at its apex. This may mean that the germ cells, which normally would produce ova, develop into male cells, but since the ear is morphologically the equivalent of a branch it may mean only an abortive attempt at the development of the branch. Since these observations were made, Professor Morgan has kindly called my attention to the splendid work of Bleringhem on maize. My results as far as they go entirely confirm Bleringhem, though the alteration in sex patency appears to be brought about by a different cause.

The case of *Lychnis dioica*, studied by Giard, is interesting. "The young is hermaphrodite, but in certain individuals the ovaries abort, in others the stamens remain rudimentary."[1] Other instances in plants are cited by Bleringhem.

These instances are sufficient to show that what seems to be only one sex may really be both sexes with one of them suppressed. Compare also Smith's studies on *Inachus*.

At the present time, the evidence available in regard to the nature of sex and its determination is very contradictory. The observations of Smith on parasitic castration in Crustacea, the experiments of Morgan with *Drosophila*, and the studies of Wilson and others indicate that the female is pure for femaleness, but that the male is a sex hybrid of maleness (dominant) and femaleness. Diametrically opposite evidence is afforded by the experiments of Doncaster on *Abraxis*, followed by experiments on other forms by several other investigators, which favor Bateson's theory of a sex heterozygous female (femaleness dominant) and a sex homozygous male. There are still other experiments which favor neither view.

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[1] The quotation is taken from Wheeler's paper.

In spite of these contradictions, sex is such a well nigh universal attribute of living beings, that there would seem to be some equally common differences between the sexes. It hardly seems reasonable to suppose that femaleness, for example, is one thing in one group and something else in another. But whatever the common difference between maleness and femaleness as such, it by no means follows that this difference of itself determines the sex of an individual. Instead, other factors may be responsible.

The observations on *Ophryotrocha*, *Inachus*, *Lychnis* and maize afford evidence of the presence of factors which control the patency of sex, so that what is really hermaphrodite may appear to be unisexual. The case of *Siline inflata*, cited by Correns from Schultze, also points toward the presence of factors which modify hermaphrodites. There are five different types, males, andromonœcious (males and hermaphrodites on the same plant), hermaphrodites, females and gynomonœcious. Moreover, according to Correns and Shull, crossing hermaphrodites and unisexual forms results in dominance of the unisexual forms.

If we assume that hermaphroditism, which is very much more common in plants and the lower animals than in the higher animals, is the primitive condition, we have a basis from which may be derived the present diverse modes of sex determination. During the course of evolution, various changes have occurred in the gametic constitution of some individuals or modifying factors may have developed, which brings about our present diverse modes for the determination of sex in the individual. That the determination of sex, even from a Mendelian standpoint, is not brought about by the simple interaction of maleness and femaleness, is shown, I think, by the deviations from equality in the sex ratios. In man, for instance, there is a slight but constant excess of males at birth, whereas on the assumption that one sex is heterozygous, the other homozygous, either equality should be expected, or that sometimes the number of female births should exceed the male births.

Though our present evidence undoubtedly favors the view that the female fowl is a sex heterozygote, it does not necessarily follow that she is therefore a potential hermaphrodite. The hermaphrodite, it is true, may be considered as a sex heterozygote, but with both sexes patent. On the other hand maleness and femaleness may each exist in the same individual in a homozygous condition, which may be expressed in Mendelian form, thus: $\frac{♂♂}{♀♀}$ forming gametes all $\frac{♂}{♀}$. This formula may represent only the primitive hermaphrodite, other formulæ serving for existing forms.

SUMMARY.

1. The castrated drake retains his secondary sexual characters, except the ability to assume the summer plumage.

2. The castrated duck (female) assumes, more or less completely, the secondary sexual characters of the drake. The change, however, is very gradual.

3. It is suggested that the female owes her color to the presence of some modifying element, which prevents the development of the male color. It is also suggested that the modifier may sometimes be responsible for sex limited inheritance.

4. Cases in which the patency of sex has changed are pointed out. This suggests that a common basis for the present contradictory evidences regarding the determination of sex may be found in the hermaphroditic condition.

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PLATE I.

FIG. 4.[1] Breast feathers from: *A*, male No. 29 in breeding plumage; *B*, occasional variant from *A*. *C*, same male; *D*, a different male, both in summer plumage. *E–H*, normal females; *E*, from female No. 26 in November; *F*, from same female in August; *G* and *H*, from two other females. *I–L*, from female No. 24, castrated; *I* and *J*, August; *K* and *L*, November; *J* and *L* were from the upper part of the breast; *I* and *K* from the lower part.

[1] The buff or reddish bands in the originals of Fig. 4, *D*, *F*, *K*; Fig. 6, *C*, *F*, *I*, *J*; Fig. 7, *D*, *E*, *F* failed of reproduction in the plates.

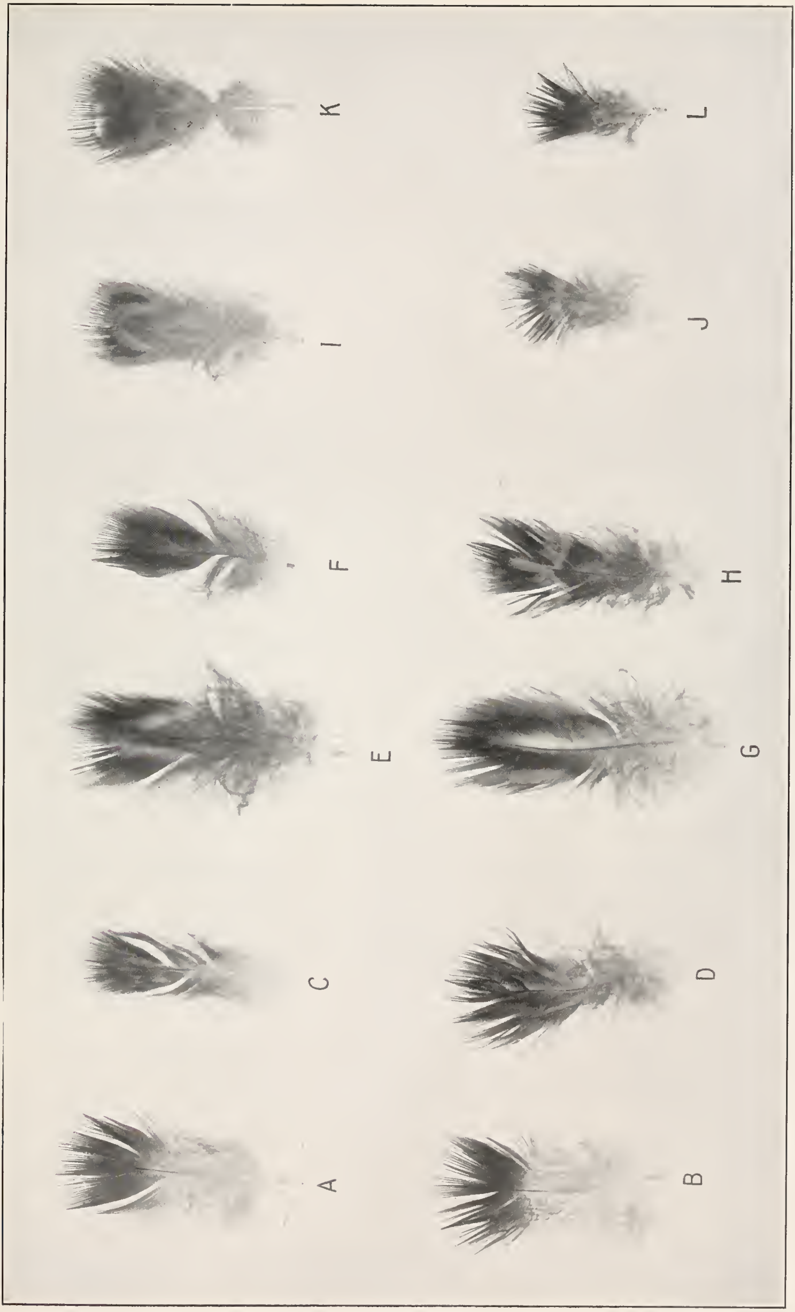


FIG. 4.

PLATE II.

FIG. 5. Feathers from near the front end of keel. *A*, *B*, 29 ♂, breeding plumage, *A* being the more common type. *C*, *D*, 29 ♂, summer plumage; *C* is usual type. *E*, *F*, normal females; *E*, 26 ♀; *F*, 41 ♀. *G–I*, 24 ♀, castrated; *G*, August; this feather from *middle* keel region; *H*, *I*, November, *H* being the common type.

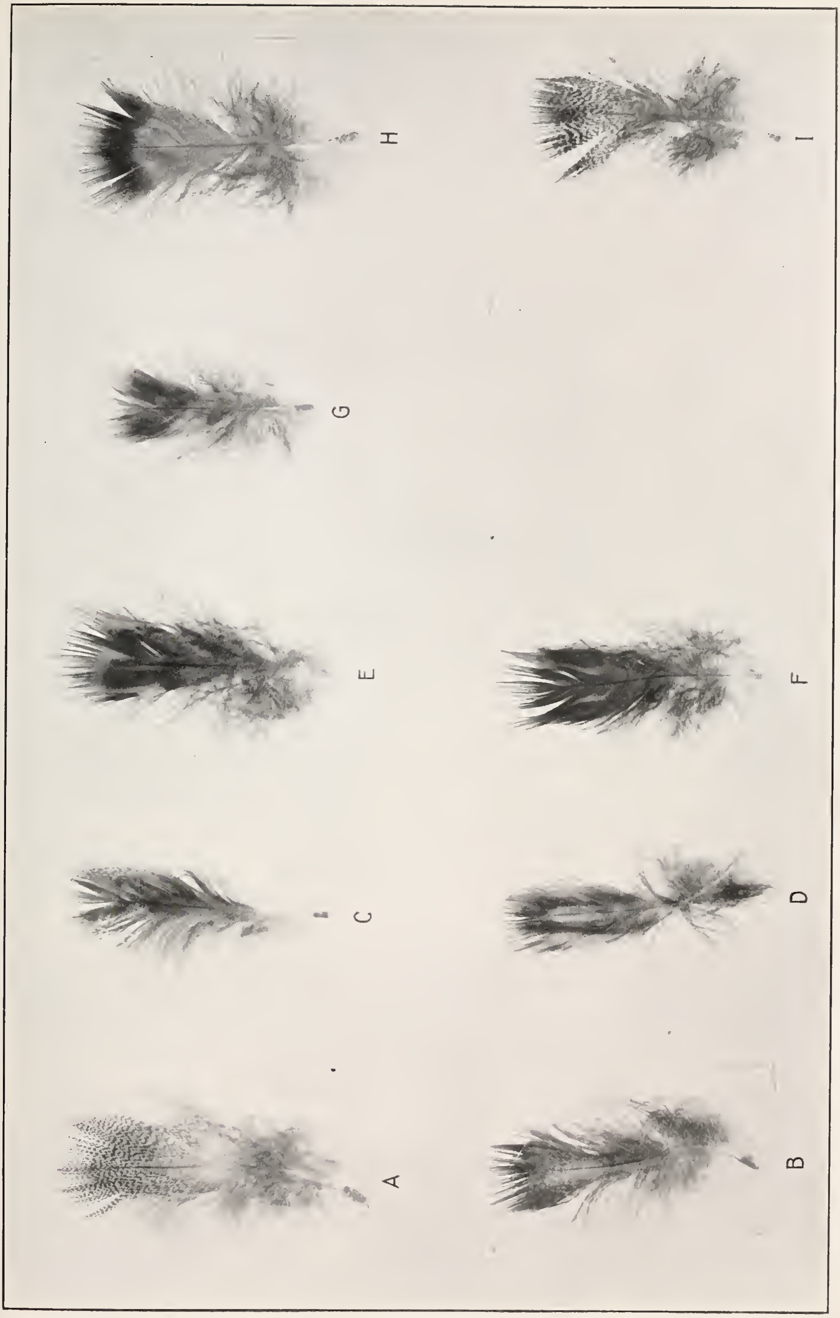


FIG. 5.

H. D. GOODALE

PLATE III.

FIG. 6. Feathers from dorsal surface near juncture of neck with body. *A*, *B*, 29 ♂, breeding plumage; *A*, common type. *C*, 29 ♂, summer plumage. *D–F*, normal females; *D*, 26 ♀, November; *F*, 26 ♀, August; *E*, 41 ♀. *G–J*, 24 ♀, castrated; *G*, *H*, August; *I*, *J*, November; *I* is more common than *J*.

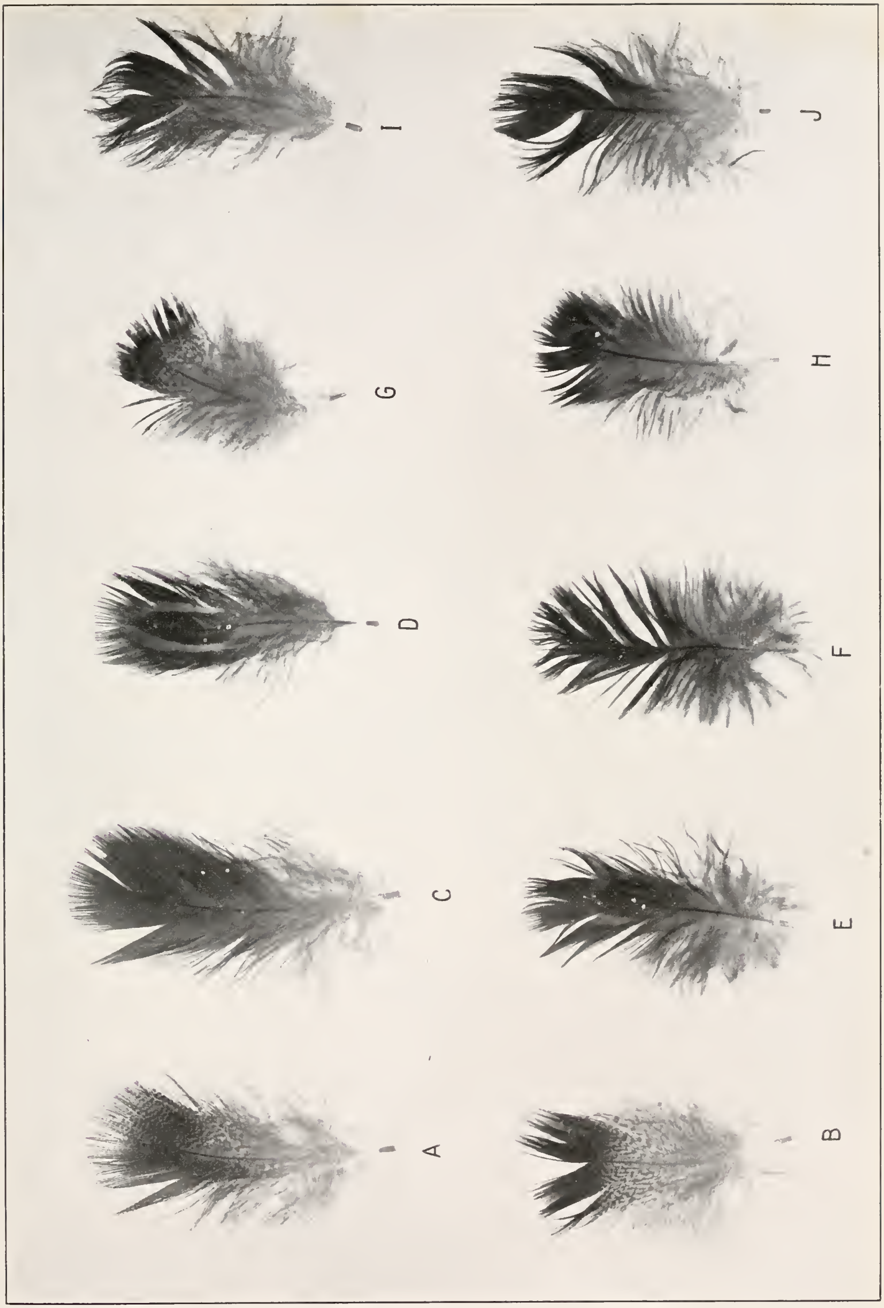
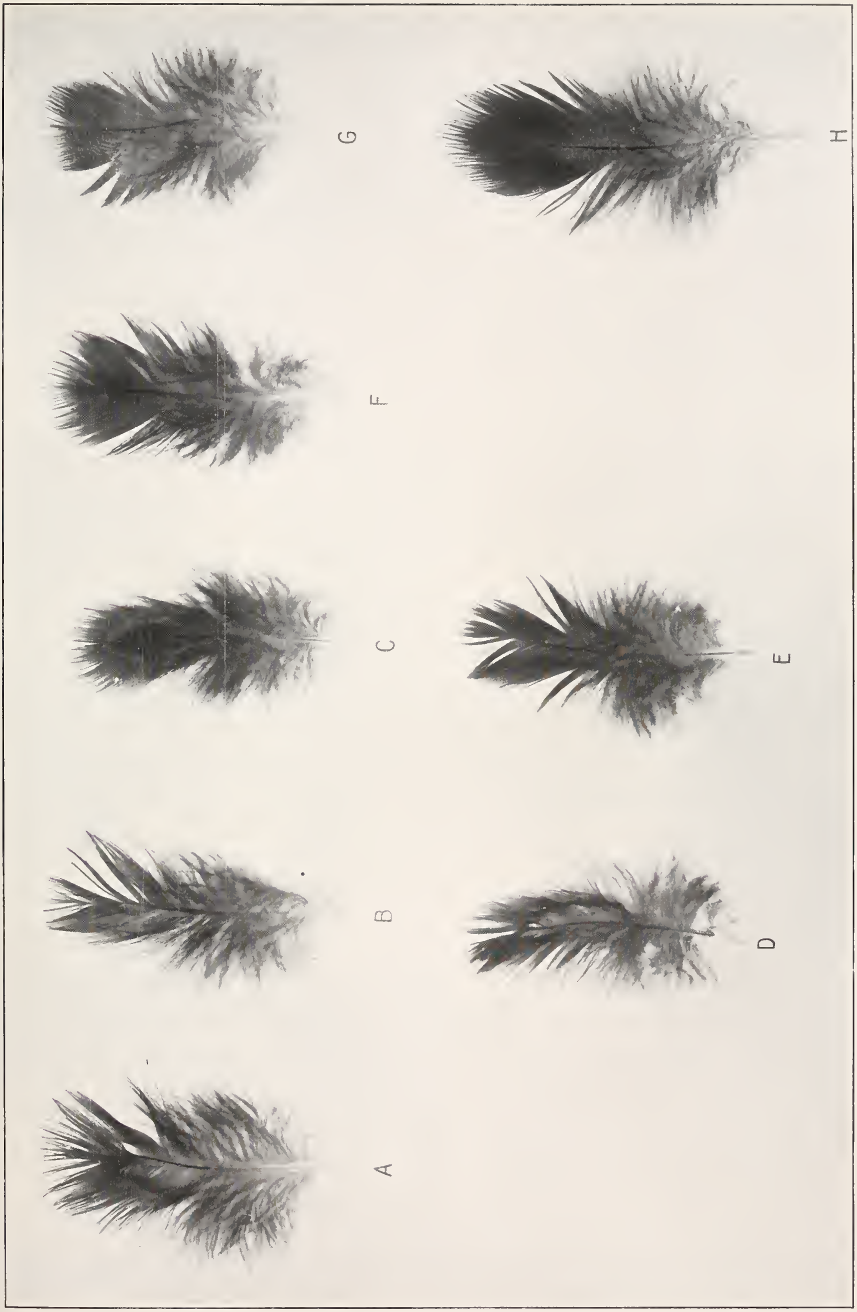


FIG. 6.

Plate IV.

Fig. 7. Feathers from rump. *A*, 29 ♂, breeding plumage; *B*, summer plumage. *C–E*, normal females; *C*, 26 ♀, November; *D*, 26 ♀, August; *E*, 41 ♀. *F–H*, 24 ♀, castrated; *F*, August; *G*, *H*, November.



H D. GOODALE.

Plate V.

Fig. 8. *A–C*, at juncture of wing and body: *A*, 29 ♂; *B*, 24 ♀, castrated; *C*, 26 ♀, normal. *D* and *E*, breast feathers: *D*, young male; *E*, young female. *G*, *I*, posterior to vent: *G*, 24 ♀ castrated; *I*, 29 ♂, breeding plumage. *F*, *H*, *J*, sides of body dorsal to vent: *F*, 24 ♀ castrated; *H*, 29 ♂, summer plumage; *J*, 29♂, breeding plumage. For normal female feathers compare Fig. 5, *E*, *F*.

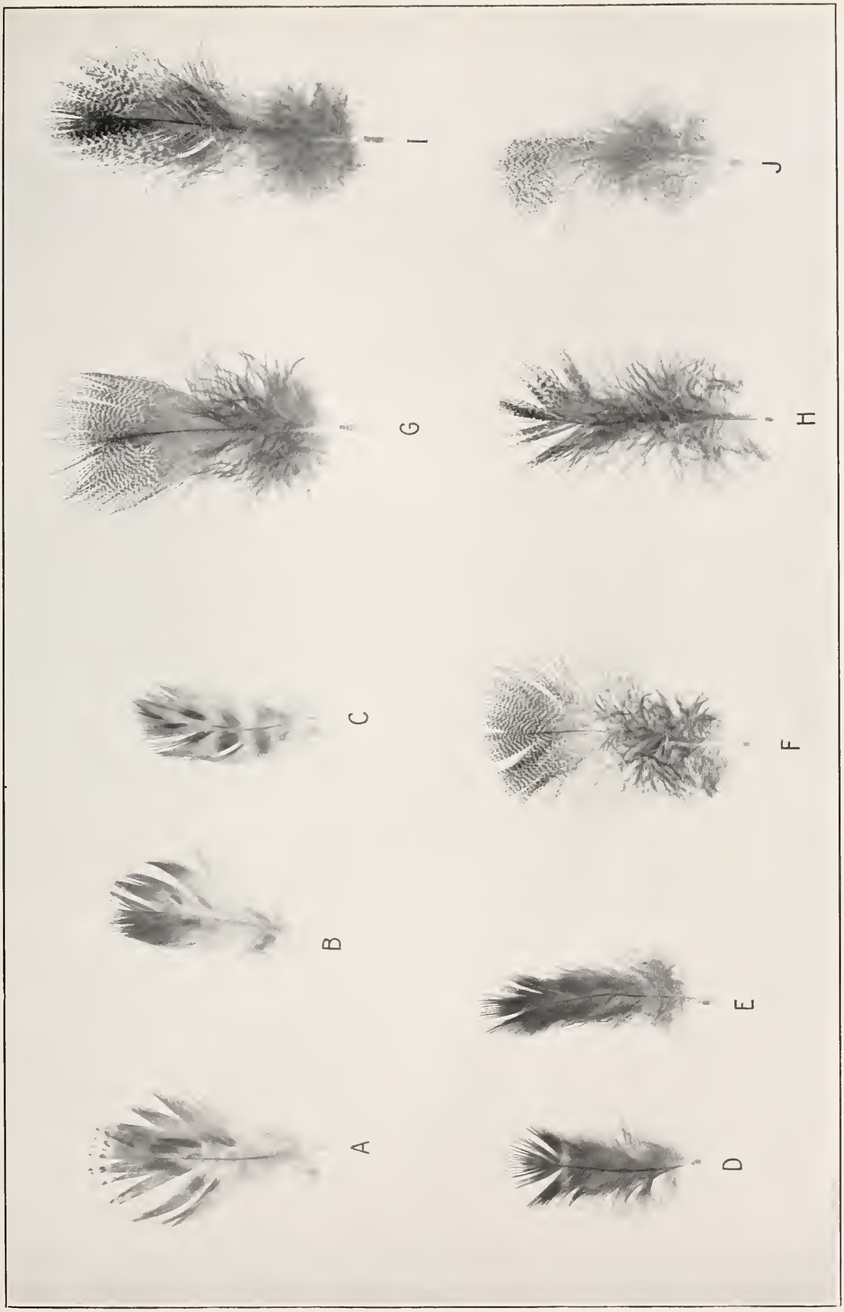


FIG. 8.

THE DETERMINATION OF DOMINANCE AND THE MODIFICATION OF BEHAVIOR IN ALTERNATIVE (MENDELIAN) INHERITANCE, BY CONDITIONS SURROUNDING OR INCIDENT UPON THE GERM CELLS AT FERTILIZATION.

William Lawrence Tower.

A Correction and Addendum.

In the preparation of the paper which appeared in this journal under the above title, certain minor experiments were taken from a larger series and combined to illustrate a general point in the behavior of alternative characters in inheritance. Through some unhappy oversight the data and plate of Ex. No. H. 410 was introduced into the printer's copy in place of the experiment intended for the position and indicated by the remainder of the paper. In this correction and addendum I have supplied the necessary data of the proper experiment with the corresponding illustration, which replaces Plate II. in the original paper. I am indebted to Professor Cockerell for calling my attention to this misplacement.

On page 294 of Vol. XVIII. of this journal, substitute the following for the account given under Ex. No. H. 410.

L. signaticollis ♀ × ♂ *L. diversa.*

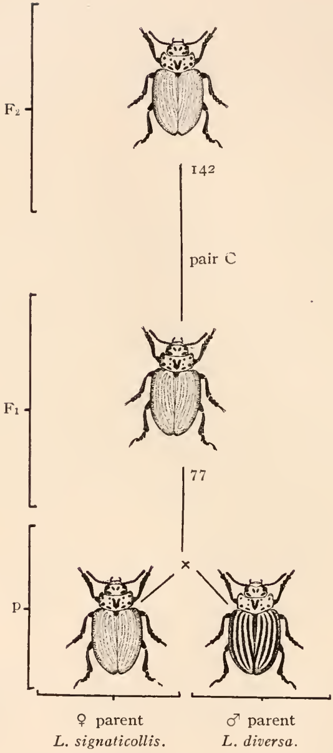
Ex. No. H. 411.

When a cross was made between a female *L. signaticollis* and a male *L. diversa* of exactly the same stocks as described in Ex. No. H. 409, under the following conditions a quite different result was obtained.

Food: normal and uniform.

	T.		R. H.
Day Av.	75° F. ± 5° F.	Day Av.	50 per cent. ± 10 per cent.
Night Av.	50° F. ± 5° F.	Night Av.	80 per cent. ± 15 per cent.

In the F_1 generation of this experiment there was only one class of adults and these were all of the female type and exhibited a narrow range of variability. These when inbred, gave in F_2



This illustration replaces Plate II. of the original paper in Vol. XVII., p. 340. Arranged to show the results obtained in crossing *L. signaticollis* ♀ × ♂ *L. diversa* under the conditions of Ex. No. H. 411. The differences in results shown between H. 410 and H. 411 are due to factors not considered in the original paper.

the same type without the least trace of separation into categories, and with the same low range of variability. When again inbred, they gave in F_3, the same type without the least trace of the attributes of the male parent stock. Altogether I have repeated this experiment six times with identical results. All have been carried to F_3 and some of them to F_5 and F_6. In the plate which accompanies this correction is shown the behavior of F_1 and F_2.

On page 295, line 25, for Ex. No. H. 410 read Ex. No. H. 411
“ “ 304 “ 3 “ “ “ “ 410 “ “ “ “ 411
“ “ 304 “ 12 “ “ “ “ 410 “ “ “ “ 411
“ “ 304 “ 14 “ “ “ “ 410 “ “ “ “ 411
“ “ 304 “ 19 “ “ “ “ 410 “ “ “ “ 411
“ “ 330 “ 12 “ “ “ “ 410 “ “ “ “ 411
“ “ 334 “ 4 “ “ “ “ 410 “ “ “ “ 411

Throughout the paper the references are to Ex. No. H. 411 instead of H. 410.

The description and plate of Ex. No. H. 410 are correct as are the conditions of experiment as far as given. This experiment, however, is from a second series of cultures parallel to the one given, but in which there are other factors involved, which in H. 410 are productive of a typical Mendelian behavior. I do not at this time care to make any statement of what these factors are, nor of their relation to the behaviors given in the H. 409, H. 411, H. 409/11 series which are the simplest and most easily presented series obtained in the crossing of *L. signaticollis* and *L. diversa*.

Department of Zoölogy,
The University of Chicago,
December 10, 1910.

Vol. XX. *January, 1911.* *No. 2*

BIOLOGICAL BULLETIN

CERTAIN HABITS, PARTICULARLY LIGHT REACTIONS, OF A LITTORAL ARANEAD.

THOS. H. MONTGOMERY, JR.

This tiny beach-comber has been identified by Mr. Nathan Banks as *Grammonota inornata* (Emert.), a species of theridiid, for which I am much indebted to him. It lives at Woods Hole, Mass., and in its vicinity, upon the sandy beaches along Vineyard Sound and Buzzards Bay. Here it is found, however, in only small scattered colonies for it occurs only on those sand beaches where the eel grass, thrown up by the sea, lies in more or less permanent lines upon the beach. Thus it does not exist upon gravelly or rocky beaches, nor yet upon sandy beaches devoid of the eel grass. The area of its distribution is limited to this cast-up eel grass, from a line slightly above high tide level landward as far as the patches of eel grass extend, a distance rarely exceeding ten or twelve feet and determined more or less by the degree of slope of the beach. In this peculiar home it obtains perpetual moisture and darkness, and does not migrate further up the beach nor into the salt marshes. In the same places occur a variety of animal forms in great individual abundance. Of this fauna the gammarids constitute the only truly marine element, the others being mainly terrestrial forms: certain other spiders, of which a drassid and the young of *Trochosa cinerea* are most abundant, staphylinid and chrysomelid beetles, an acarine, a pseudoscorpion and a chilopod. These are all normal constituents of this fauna, not waifs drifted in by the sea.[1]

My interest was drawn to them by the observation that when they are disturbed by the removal of the eel grass, they all run directly landward provided only the sun is shining from an angle. Fifty or a hundred or more of these spiders may be set into commotion by the lifting up of a bunch of the eel grass, and under the conditions stated the large majority, sometimes all, of them invariably run in a straight course right up the beach and do not stop until they reach shelter beneath another mound of the eel grass. This habit is the more striking because none of the other species that live with them exhibit any such regular landward migration.

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[1] The elements of the "upper beach fauna" have been interestingly characterized by Davenport, "The Animal Ecology of the Cold Spring Sand Spit," Dec. Publ. Univ. Chicago, 1903. But he does not mention the particular species we are now considering.

Direction and velocity of the wind have nothing to do with determining this course of running, for the landward migration occurs whether the wind blows upon the spiders from in front, or behind or from the side, or when there is no wind. Further, the spiders are not guided by any moisture sense, for (1) they do not regularly run landward when the sun is obscured, and (2) if the observer pours out sea water at a higher level and lets if flow down towards the spiders, they nevertheless continue their landward course. Again, this is not a geotropism, a tendency of the spiders to run up hill, nor an orientation to the rolling of sand grains. For the observer can dig a deep trench to landward of the spiders, and they will go down its declivity and up its opposite side without changing their course; or one can implant a board vertically in the sand in front of them, when they will climb up one surface of it and down the other without changing their general direction of movement.

Therefore the landward course of these spiders is not caused by any influence of wind or moisture or slope of the beach, but is probably a light reaction as the following observations would show.

When the sun is shining and when its rays come from an angle, at any time but noon, the spiders regularly run up the beach; this I have tested many times and on different beaches facing different points of the compass. That is, the spiders run up the beach whether the sun shines in front of, behind or to one side of them. But at noon, when the sun is nearly vertical, they run in all directions; and that they do also when the observer on a sunny day shades them with an umbrella.

Then a number of experiments were made to test the reactions of the spiders to the rays of an ordinary student oil lamp placed one foot or a foot and a half away from them, all these experiments being made at night. Numbers were caught and placed in small vials, which can be done without injury to them for they are hard-bodied. Then a piece of smooth white paper was laid horizontally upon the table, and kept free from sand or other particles; on this paper four quadrants were marked, the one nearest the lamp marked *A*, the one most distant from it *D* and the other two quadrants *C* and *B*. A vial was then uncorked and the spiders allowed to drop in succession gently upon the center of the paper, each falling slowly on its own drag line. With a pencil the course of each spider was then marked upon the paper, which made a permanent record of all movements of all individuals. On different nights a total of 107 spiders were thus tested, with the following results: 3 ran into quadrant *A*, directly towards the light; 45 ran into quadrant *D*, directly away from the light; 28 turned into quadrant *B* and 31 into quadrant *C*, these accordingly at right angles to the direction of the light. This proves negative phototropism to the rays of an oil lamp, for only 2.8 per cent. of the individuals ran towards the light. In these experiments it was purely a matter of chance how the spider was oriented when it first touched the paper. Of those that happened to touch it facing the light almost all moved through an arc of a circle so as to get into another direction and then continued their courses in almost straight lines; there was a marked turning away from the source of the light.

Another experiment was made with diffuse daylight. Eleven spiders were dropped in similar manner upon a piece of white paper placed on a table in the northwest corner of a room, sunlight entering the east window but not reaching the table. Ten of the spiders turned directly away from the sunlight, and one at right angles to it. This was then negative phototropism.

In a third experiment spiders were dropped upon a piece of white paper on which the sun shone directly, the sun being then near the meridian (10.45 A.M.). In this case ten moved towards the sunlight, one at right angles to it, and eight away from it.

This result seems at first sight to be contradictory to the preceding experiments. But I believe it is explainable by the nearly vertical position of the sun's rays; for on the beach, at noon when the sun is nearly vertical, the spiders do not run in any regular direction.

It thus seems that these spiders are decidedly negatively phototropic to lamplight and to diffuse daylight, when these impinge upon them from an angle; but that when sunlight falls upon them nearly vertically they do not orient themselves to it. How is it then on the beach that the spiders run so regularly landward whenever the sun is shining from an angle, irrespective of the position of the sun, which may be in front or behind or to the right or the left of them? One would not suppose it to be a case of negative phototropism when they run straight towards the sun. Yet I believe it is this nevertheless, in that the spiders may orient themselves not to the sun directly but to the light from the water. For the area of light in the sky and clouds above the water is certainly greater, and the light more intense, than that to landward, owing to the great amount of reflection from the water. It is well known that a man becomes more sunburnt upon the water, a test that the sunlight is more effective there. It would seem that only in this way can we correlate the experiments made with the oil lamp and with diffuse sunlight, with the observations recorded upon the sea beach. That animals react to area as well as to intensity of light is now well known. Thus G. H. Parker[1] showed that *Vanessa*, when in the open, always comes to rest with its head away from the sun, and that it thereby "reacts positively to large patches of bright sunlight rather than to small ones, even though the latter, as in the case of the sun, may be much more intense." Cole[2] has confirmed Parker's results, and concluded from his own painstaking observations on several forms, that animals with direction eyes, as those without eyes, respond to light intensity only; and that animals with image-forming eyes when positively phototropic react to the size of the luminous field or to definite objects in the visual field.

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[1] "The Phototropism of the Mourning Cloak Butterfly, *Vanessa antiopa* Linn.," Mark. Anniv. Vol., 1903.

[2] "An Experimental Study of the Image-forming Powers of Various Types of Eyes," *Proc. Amer. Acad. Arts and Sci.*, 42, 1907.

Our *Grammonota* probably possesses an image-forming eye, but it is negatively phototropic. Whether it reacts to intensity of the light or to its area remains to be determined. I have made no attempt to decide whether it reacts rather to the light rays than the heat rays, and I was not interested to determine this, for in the state of nature heat and light, so far as they affect these animals, are probably always associated.

Under natural conditions the masses of eel grass that shelter these spiders would be disturbed only by unusually high tides, perhaps occasionally also by violent winds. If the sun were shining during such a disturbance the spiders, from their negative phototropism, would attempt to run landward, and thus some of them might escape the action of the waves; but it is questionable whether this light reaction would be of any great benefit to them at times when the waves play havoc on the beach.

From the scattered distribution of these spiders one would suppose they might be readily transported by water. I accordingly made a few experiments to ascertain how sea water affects them. A number were dropped upon clean sea water placed in a glass dish; they were able to stand upon the surface film, but not to run unless there were fine dust particles upon it. But so soon as the water was violently agitated they sank below the surface and were unable to rise again. Therefore they could not long remain upon the top of a wave, but would become quickly submerged unless they clung to some floating vegetation. This is because the body is only slightly pilose. They become quiet after a few minutes of submergence, as though partially suffocated, yet they can withstand submergence for some hours. Thus I kept seven beneath water for five and a half hours, then placed them upon blotting paper to dry, when four revived; and I kept another lot beneath water for sixteen hours, and one of these revived after drying.

A number were placed in isolated vials, each with a few drops of sea water to furnish the necessary moisture, in order to observe the cocooning, but I was not so fortunate as to see this process. The cocoon is lenticular, snow-white and relatively very large—its diameter considerably greater than the length of the spider. Nine cocoons were made in these vials, by as many females, and all at some time between 10:30 P.M. and 7 A.M., the time of cocooning would then seem to be nearly morning. On previous occasions I have indicated how regular this time is in spiders, and how it varies with different species.

Males are abundant through the summer months, but the mating was seen only in part although many of both sexes were kept in observation cages. A male and female were seen to move directly towards each other, and on meeting each elevated its head region so that the line of the body made an angle of about 45° with the floor, when the male extended his cephalothorax somewhat beneath and to one side of that of the female. This attitude is similar to that of *Dictyna*.

UNIVERSITY OF PENNSYLVANIA.

PRELIMINARY NOTE ON THE ECOLOGY OF THE EARLY JUVENILE LIFE OF THE UNIONIDÆ.[1]

F. B. ISELY.

During the past four years the writer has given considerable time to the field study of the Unionidæ. A number of puzzling questions have arisen as to the relative importance of the various ecological factors that contribute to the environmental complex of these animals. Among the most important of these factors, we may mention bottom conditions, water content, stream volume, stream fluctuations, relation to fish and natural enemies.

In connection with this work, much difficulty was experienced in finding young mussels for study and experimentation. I have collected many specimens from the size of a nickel to a quarter, but mussels under the size of a dime have been rare. A number of experienced field workers have spoken to me of a similar difficulty in finding juvenile specimens.

In order to avoid any misunderstanding, I may state that by "early juvenile life" I mean the period following the time when the mussel completes the parasitic stage and leaves the fish to lead an independent life, until it is about the size of a dime or about fifteen millimeters in length. This would cover, in most species, approximately the first year of independent existence. Other periods may be designated as later juvenile and adult life.

The glochidium stage has received considerable study. Our knowledge of the parasitic period has been recently cleared up and extended by the careful investigations of LeFevre and Curtis.[2] The later juvenile and adult life has received the attention of hundreds of students. The scattering and meager references in the literature, to the early juvenile stages, give us little information on this important period in the life history of the mussel.

During the past summer, while working on the Red River Mussel

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[1] Published by permission of the U. S. Commissioner of Fish and Fisheries.

[2] "Reproduction and Parasitism in the Unionidæ," George LeFevre and Winterton C. Curtis, *Journal of Experimental Zoölogy*, Vol. IX., No. 1, September, 1910, pp. 79–115.

Survey for the Bureau of Fisheries, I found a fairly good number of species in the early juvenile period of development. The distribution and ecology of these young mussels seem to me to be of special interest. Furthermore, the facts in regard to their habitat seem to clear up some ecological perplexities concerning the adult ecology of the Unionidæ.

Thirty-two specimens are here especially considered, these were attached to rocks and pebbles by a functional byssus. These mussels, representing nine species, were found in three different rivers and in four localities. The situations, however, were similar. Twenty-nine of these specimens weigh less than five decigrams; of this number twelve weigh under one decigram, and ten are under nine millimeters in length.

The thirty-two specimens represent the following species:[1] (1) *Lampsilis luteola*, two; (2) *Lampsilis fallaciosa*, one; (3) *Lampsilis parva*, four; (4) *Lampsilis gracilis*, three; (5) *Plagiola elegans*, one; (6) *Plagiola donaciformis*, sixteen; (7) *Anodonta imbecillis*, two; (8) *Ptychobranchus phaseolus*, two; (9) unnamed species, one.

The first of these specimens was secured on August 20, 1910, in the Kiamichi River, near Fort Towson, Oklahoma. While working in a gravel bed, Owen Horne, one of our party, called attention to an unusually small mussel attached to pebbles by a byssal thread. After a search for about two hours we secured eight specimens with byssus, representing four species. These specimens were found in a coarse gravel bed, the pebbles being from one fourth to one inch in diameter. The water was fairly swift, and from one to two feet in depth. The byssus of these specimens was variable in length, sometimes several inches long, and often connecting three or four small pebbles. Sometimes the byssus spread into several branches at the place of attachment. The young mussels were best secured by taking up handfuls of gravel and looking for the thread. The byssus is strong enough to support the mussel in a rapid current, and will sustain the weight of a number of small pebbles, without breaking.

On August 30, 1910, five more specimens were secured in the

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[1] Most of the specimens have been examined by F. C. Baker and L. S. Frierson, making sure of the identifications.

Little River, near Garvin, Okla. One of these specimens, found by E. C. Johnston, of our party, was attached by the byssus to the shell of a large *Quadrula pustulosa*. An observation of this kind is described by Kirtland.[1]

During the afternoon of August 30, we again investigated a portion of the Kiamichi River, near Roby, Okla. Here we secured fourteen specimens; ten of these I secured in about half an hour, one time bringing up three specimens with a handful of gravel. Here again the environment was typical, fairly swift water, coarse gravel, and rocks.

A search for young mussels was also made in Blue and Boggy rivers, but failed to yield specimens with functional byssus. However, a number of mussels under twelve millimeters were secured, representing *Quadrula pustulosa*, *Quadrula lachrymosa*, *Quadrula coccinea*. In the Washita River, near Davis, Okla., on September 2, we secured the last of our young mussels for the summer. Five specimens were found in swift water about two feet deep. One of these was under three millimeters in length and weighed .005 of a gram.

In the following table I show species, locality, weight in grams, length, height, and breadth in millimeters, of ten of the thirty-two specimens:

Species.	Weight.	Length.	Height.	Breadth.	Locality.
1. *L. luteola*	.41	14.5	9.2	5.9	Kiamichi R., Aug 30, 1910.
2. *L. fallaciosa*	—	13.5	6	3.4	Kiamichi R., Aug. 20, 1910.
3. *L. parva*	.035	7	3.8	2.3	Kiamichi R., Aug. 20, 1910.
4. *L. gracilis*	.47	22.5	9.9	4.1	Little R., Aug. 30, 1910.
5. *A. imbecillis*	.055	7.1	3.5	1.1	Kiamichi R., Aug. 30, 1910.
6. *P. phaseolus*	.15	12.4	5.6	2.7	Little R., Aug. 30, 1910.
7. *P. elegans*	.175	9.6	6.9	4.5	Kiamichi R., Aug. 30, 1910.
8. *P. donaciformis*	.005	2.9	1.5	1.1	Washita R., Sept. 2, 1910.
9. *P. donaciformis*	.01	5.6	3	1.9	Kiamichi R., Aug. 30, 1910.
10. Unnamed sp.	.014	5	3.3	2.3	Little R., Aug. 30, 1910.

The facts noted above are closely related, not only to the ecology of the juvenile mussel, but also to the ecology of the adult.

1. They indicate the conditions essential for the most successful growth and early development of the Unionidæ. This kind of

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[1] "Fragments of Natural History," J. P. Kirtland, *American Journal of Science*, Vol. 39, 1840, pp. 166–168.

an environment gives a constant supply of oxygen, and sufficient food; is frequented by suitable fish; is free from shifting sand and silt accumulation. Those mussels that drop from the fish in these favorable situations develop in large numbers, while the less fortunate, that drop in shifting sand and silt, die early.

2. In the study of the ecological factors that are inimical to mussel life, more attention should be given to the consideration of the juvenile habitat. Absence of gravel bars and stony situations may sometimes explain the scarcity of the Unionidæ in certain streams and lakes where frequently water content has been thought the chief unfavorable factor.

3. It is a well known fact that in many streams certain stretches of mud-bottom are found loaded with mussels, while other areas, in the same stream, equally favorable from the standpoint of the habitat of the adult mussels, have only scattering specimens.

This distribution of the adult may be explained by the assumption[1] that the average mussel seldom travels far up or down the stream from the place where it begins successful development. Stretches favorable for juvenile development thus come to be the centers of dispersal in the streams where they occur. As a result, areas of mud-bottom near these favorable habitats, become loaded with mussels by migration.

4. In the study of the life history of the Unionidæ, we may consider the embryonic, the glochidia, the parasitic, the *early juvenile*, and the adult as distinct periods for separate and special study.

University Preparatory School,
Tonkawa, Oklahoma.
November 25, 1910.

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[1] This assumption is fairly well established, by experimental study that I have been carrying on for some time, and will be discussed in a later paper.

A STUDY OF PEDAL LACERATION IN ACTINIANS.

LEWIS R. CARY.

Since the appearance of Andres's ('82) classical paper "Intorno alla Scissiparita della Attinæ," the only accounts of the process of pedal laceration have been very casual, or have been given only for the sake of comparison with the phenomena of induced regeneration. Carlgren ('04), ('09), who alone has studied this form of reproduction in the genus *Aiptasia*, makes mention only of the sequence of the appearance of the mesenteries and tentacles, but gives no general account of the process, nor does he mention any of the histological features.

Since the last-mentioned phase of the subject is of considerable interest as a comparison with the development of normal embryos, it has seemed advisable to publish the following account to supplement the previous contributions to the knowledge of this type of reproduction which is so common among the actinozoa.

The observations herein recorded are based upon the study of four species of actinians: *Aiptasia pallida* Agg., *A. tagetes* D. & M., *A. annulata* Andres and *Cylista leucolena* Agg.[1] Of these species *A. pallida* and *Cylista* were obtained in abundance at Beaufort, N. C.,[2] during the summer of 1903 and 1904, where they were studied both in their natural environment and in aquaria in the laboratory.

Specimens of the first-mentioned species were transferred to Baltimore and kept during two years in aquaria in the zoölogical laboratory of the Johns Hopkins University. By means of the diatom method described by Dr. Caswell Grave for rearing echinoderm larvæ, the actinians were kept in good condition during this time and they continued to reproduce by laceration throughout the year, although no embryos were ever set free during either of the breeding seasons.

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[1] Professor McMurrich informs me that he has conclusive evidence that the form described by Verrill from Beaufort, N. C., as *C. leucolena* is not the *C. leucolena*, of A. Agassiz, but in the absence of any published statement to that effect Verrill's terminology will be used.

[2] I am indebted to the officials of the U. S. Bureau of Fisheries for the use of a table, and equipment at their Beaufort, N. C., laboratory during the two seasons above mentioned.

The specimens of *A. tagetes* studied were obtained in 1909, during a stay at the Bermuda Biological Station.

A. annulata was obtained during the past summer while the writer was at the laboratory of the Carnegie Institution of Washington at Dry Tortugas, Florida.

The two last-mentioned forms were studied in aquaria in the laboratory only.

Material of the four species was preserved, after being narcotized in magnesium sulfate, in a number of fixing reagents; of which Petrunkewitsch's fluid and Bouin's fluid gave the best results. All of the *Aiptasias* cut readily after being imbedded in paraffin in the usual manner, while the mesoglea of the *Cylistas* proved so refractory that double imbedding in celloidin and paraffin was necessary in order to secure an unbroken series of sections.

In common with the tissues of most actinians, many of the hæmatoxylin stains were highly selective, staining the ectoderm much more heavily that the endoderm so that by using an alcoholic solution of eosin as a counter stain after Delafield's hæmatoxylin there resulted an absolutely certain color differentiation between the two tissues by means of which any question of the origin of a certain structure could be determined by its staining reaction.

General Account of the Process of Laceration.

As has been described by Andres (1882), the beginning of the process of laceration is characterized by a certain part of the base of an actinian becoming very firmly attached to the substratum while the animal as a whole moves away from the point which is thus relatively immovably attached. As a result the tissues about this point become strongly stretched and attenuated. With the continuation of the contraction, there finally comes a rupture of the tissues at some little distance from the free border of the disc so that the resulting laceration piece has usually an appearance such as is shown in Fig. 1, Plate I., which represents a fragment from the base of a specimen of *A. pallida* immediately after its separation from the parent individual. The *acontia*, which in laceration pieces of this form are at first so prominent, are later either withdrawn inside the cavity of the fragment, or else they are broken off before the open side of the piece has closed to any considerable extent.

Immediately after the separation of the fragment there takes place a rolling-in of the free edges, due principally to the elasticity of the mesoglea, so that for some little distance within the cavity is lined with ectoderm (Fig. 7, Pl. II.). For some hours after the separation of the fragment there is no visible external change other than an increasing loss of color about the orifice of the tear where the laceration piece was separated from the parent individual.

The next noticeable change appears as a turning upward of that portion of the fragment in which is situated the original opening, which by this time has become markedly reduced in extent; although, contrary to the results of Hazen (1902), who worked with fragments obtained by cutting off pieces including portions of the column wall and pedal disc from adult specimens of *Sagartia lucæ*, in none of my specimens did the opening become entirely obliterated. The new growth which brings about this change in the shape of a laceration piece is much the more rapid on the lower-pedal disc-side of the aperture. So that for some time the elevated area is situated near the former internal edge of the fragment. The wall that has come from the up-growth from the pedal disc is nearly perpendicular, while the opposite side of the fragment slopes away much as in its former manner (Fig. 2, Pl. I.).

In a stage of development such as is represented in Fig. 2 there has already appeared the beginning of the tentacle buds, and internally, of course, the earliest mesenteries.

In a later stage, as in Fig. 3, Pl. I., the readjustment of materials had proceeded farther in what is practically the same course. The fragment as a whole has become much higher. The oral end has nearly the same diameter, while farther down toward the pedal disc the diameter is relatively much decreased. At the base, however, the fragment still covers practically the same area as when it was torn off from the parent animal. When viewed from the side, as in this figure, the lines corresponding to the insertion of the old mesenteries are seen to extend only a short distance upward from the base and to be less distinct toward that side which is most nearly perpendicular, *i. e.*, which has arisen from the tissue which was originally part of the pedal disc. The tentacle buds have increased very slightly in size, but at the oral end of the fragment, which now has the appearance of a partly contracted *Aiptasia* there can be made out through the almost transparent column wall, the outlines of the stomodeum and the lines of insertion of the developing mesenteries.

Figs. 4 and 5 were, in order to show the arrangements of the tentacles, drawn in a nearly oral view, so that the upper part of the column is not visible. The fact that the lines of insertion of the old mesenteries appear to extend nearly the whole length of the column is thus to be explained by the foreshortening in the figure and not by any difference as regards the mesenteries between these specimens and that shown in Fig. 3. In the specimen shown in Fig. 4, there are eight tentacles, of which one is considerably longer than the other seven, all of which are of approximately the same length. In practically all instances one tentacle outstrips the others in its development so that for a considerable time it may be distinguished from the others. On the other hand there seems to be no such definiteness in the determination of just what tentacle shall first appear as there is in many actinian embryos where the tentacle above the endocœle between the two dorsal directive mesenteries almost invariably makes its appearance first.

In later stages there is often a very marked difference in the sizes of the tentacles of the first series, as in the specimen represented in Fig. 5. Here a single tentacle has far outstripped the others, while of the remaining seven three are decidedly larger than the remaining four. In Fig. 5 the tentacles of the second set are seen to be arising in pairs at each side of several of those of the first set. While that tentacle of the first set which is uppermost in the figure is by far the longest, there is on either side of it a single short tenacle of the first set, and no indication of the paired tentacles of the second series. The tentacles on the opposite side of the disk have already acquired their definite shape and appearance.

The young actinian shown in Fig. 6 has already acquired the characteristic appearance of all small specimens of this species, so that, from an external examination, it would be impossible to determine whether it had come directly from an egg or from a laceration piece. As compared with any of the figures of earlier stages the proportionate diameter of the column is very much reduced. This has been brought about by a continuation of the same processes that are shown at an earlier stage in Fig. 3. The basal portion of the column is now of no greater diameter than the upper part, while the whole animal has become considerably elongated. The first eight tentacles are now of practically the same length as are also the eight pairs of secondary tentacles. Internally the stomodeum can be seen to have increased greatly in length. The mesenteric filaments, not previously distinguishable, have now become very prominent structures on the first eight mesenteries. The column wall has become thin and has lost its pigment clear to the base through which are seen the points of insertion of the first eight mesenteries, while those mesenteries that came over from the parent actinian in the laceration piece, have entirely disappeared, even at the extreme base of the young specimen.

In following through the development of any single laceration piece from the time of its separation from the parent until it becomes a typical young actinian it is apparent that there has been very little, if any, increase in its bulk, and that the change in shape has come about almost entirely through the readjustment of the materials already present and not to any considerable extent through the metabolism of new material.

Frequency of Reproduction by Laceration among the Forms Studied.

The following table shows the frequency of this method of reproduction among the four species studied and the number of young that has arisen from a single parent individual within a given time.

In another instance many thousand individuals of *A. pallida* were examined casually, but without any records being kept, and here again it was found that the percentage of those which had undergone laceration was very high. In fact this form of reproduction was much more frequent in the last-mentioned instance—on the government jetties at Cameron, La., than in the case of the same species from which the records were made at Beaufort.

Species.	Number Examined.	Number that had Given Rise to Laceration Pieces.	Number of Laceration Pieces.	Locality.
C. leucolena	300	292	11.32 (0–30)	Beaufort, 1903.
C. leucolena	1,000	942		Beaufort, 1904.
A. pallida	150	78	6 (0–14)	Beaufort, 1904.
A. tagetes	220	187	10.4 (0–26)	Bermuda, 1909.
A. annulata	52	20	3 (0–7)	Tortugas, 1910.

INTERNAL CHANGES DURING LACERATION.

Under this head will be discussed the following topics:

(*a*) The formation of the stomodeum.

(*b*) The development of the mesenteries.

(*c*) The development of the mesenteric filaments and acontia.

(*d*) General histological changes in the tissues during development.

(*a*) *The Formation of the Stomodeum.*

As mentioned previously in the general account of the process of laceration, the first noticeable change in the appearance of a laceration piece after its separation from the parent consists in the rolling in of the edges of the torn side of the fragment, so that from the first the opening into the cavity of the fragment is lined for some distance with ectoderm (Fig. 7, Pl. II.). At first the ectoderm extends forward for only a short distance on the upper side of the opening, while on the lower side the inrolling has progressed considerably farther.

In a section through a stage such as is shown in Fig. 2, Pl. I., the stomodeum has the appearance such as is shown in Fig. 8, Pl. II. Here the free edges about the orifice of the fragment have become thinner, the mesoglea is reduced to a thin sheet of tissue and the cavity of the stomodeum has become vertical instead of nearly horizontal as at first. At the lower, free, border of the stomodeum the ectoderm has become turned outward and extends around the border of the endoderm onto the internal wall of the stomodeum.

In Fig. 9, Pl. II., which represents a section through a stage slightly older than that shown in Fig. 3, Pl. I., the stomodeum has reached nearly its normal length and, on the left side in the figure, its ectodermal lining is shown extending down along the border of a mesentery to form the mesenteric filament. On the opposite side where the section passes through the chamber between mesenteries, the ectoderm forms a flat plate extending across the free border of the stomodeum.

(*b*) *The Development of the Mesenteries.*

Andres (*loc. cit.*), in his figures illustrating the rearrangement of the mesenteries in the young fragments, shows only the pedal disc and apparently understood that the mesenteries which come over in the laceration piece from the parent were rearranged to become the permanant mesenteries of the young actinian. He makes no mention of any new growth of mesenteries or stomodeum at the oral end of the fragment as it becomes elongated and assumes the characteristic shape of an actinian.

Carlgren, 1904, 1909, takes up to a considerable length the question of the arrangement of the mesenteries in laceration embryos. He distinguishes four types of arrangement of the first twelve mesenteries in such embryos: depending both on the sequence in the appearance of the mesenteries, and on whether or not any of these mesenteries, are situated in old tissue. "Was erstens die Entwicklungstypus der Mesenterien in den Lacerationstückchen anbelangt, so haben die 1904 und die hier oben mit geteilten Untersuchungen gezeugt dass es verschiedene Variationen derselben giebt. Wie gross die Variation auch ist, so kann mann doch deutlischerweise in dem Lacerationstückchen, d. h. in solchen Stückchen, die einen Teil der Fussscheibe und einen der Korperwand umfassen (Text Fig. 1, *b*), drei Verschiedene Haupttypen für die Mesenterien Entstehung unterscheiden, und zwar erstens einen *bilateralen* mit *drei* primären, vollständigen, gleich, orientierten Mesenterienpaaren (II., 4.), Zweitens ein *bilateralen* mit nur *zwei* ebenfalls gleich (II., 1), angeordneten Paaren und schliesslich drittens einen *birädialen* (II., 5 and 8), welche letzteren zwei Neubildungszonen enthält, die in ihren am besten entwickelten Gestalt (No. 8), Spiegelbilde zu einander sind, indem jede aus zwei bilateral angeordneten, vollständigen Mesenterienpaaren besteht." Besides these three types Carlgren recognizes a mixed type which cannot be put in any one of the above-mentioned classes.

All the specimens of *Aiptasia* studied by Carlgren fell, with three exceptions which were very irregular in their growth, into his group three, the biradial type, in which there are two zones of new growth. All of the specimens of this genus that I have sectioned show this arrangement of the newly formed mesenteries, which, thus, seems to be characteristic of the genus *Aiptasia*. The specimens of *Cylista* also showed the same arrangement of the first mesenteries.

H. V. Wilson, 1888, states that in *Macina areolata* the œsophageal invagination is at first centrally placed in the larva, but that soon "it begins to travel toward one side of the larva." This lateral motion of the stomodeum continues until that organ comes to lie close against the outer wall of the larva. Finally as the lateral movement progresses—from above downward—the endoderm is pushed before it until the two ectodermal layers, of the stomodeum and of the body wall, are in contact, or separated only by the thin layer of mesoglea secreted between them. Soon following this stage, . . . "the œsophagus has moved away from the surface ectoderm, but while doing so has remained connected with it by a band of supporting lamella." The mesentery arises by the endoderm growing up into the space between the stomodeum and the outer wall of ectoderm as the stomodeum is drawn toward the side opposite the first mesentery, for the formation of the second one.

Appellöf, 1900, mentions the same sort of displacement of the stomodeum previous to the formation of the first mesentery. He states, on the other hand, that: "Die exzentrische Lage des Schlundrohres ist in allen Entstehungsphasen desselben konstant und deutlich nachzuweisen und ist—so nehme ich wenigstens an—auf ungleichseitiges Wachstum der Larve zurückzuführen."

In laceration embryos the stomodeum is from its first appearance excentric in position. As the change in the form of the piece takes place so that the original orifice, where the piece was torn off from the parent, becomes carried up toward the highest part of the fragment and becomes smaller and more rounded, the stomodeum on that side which has come from the growth of the pedal disc comes into contact with the body wall. The first mesentery makes its appearance at the point where the forming stomodeum is in contact with the column wall; although in none of my sections is there evidence of any marked displacement of the endoderm such as would be necessary to bring the two mesogleal surfaces into contact. As is always true in the formation of a mesentery, the growth of the mesogleal lamella here begins at the extreme oral surface and proceeds downward along the stomodeum and column wall. In laceration embryos the endoderm is carried down on both sides of the mesogleal lamella so that from its earliest appearance, the mesentery is structurally complete. As the growth leading to the acquisition of the characteristic actinian shape of the laceration piece goes on the stomodeum becomes situated more centrally in the oral disc. While this adjustment is taking place the first mesentery becomes lengthened in the horizontal direction to keep pace with the change in the position of the stomodeum.

The method of the formation of all those mesenteries formed after the stomodeum has become central in position is the same. The first indication of their appearance is seen as a slight ridge of mesoglea extending aborally from the oral disc at the point where the latter joins the column wall. As the growth of the mesentery continues the mesogleal lamella extends farther from its point of origin, both horizontally along the oral disc and proximally along the column wall. When by its horizontal growth the mesogleal lamella comes in contact with the mesoglea of the stomodeum a rapid downgrowth of the mesentery takes place. Throughout the development of a mesentery the growth is the most rapid where there is contact between the growing mesogleal lamella and that of the body wall, oral disc, column wall or pedal disc. In all such mesenteries the growth of the endoderm keeps pace with that of the mesogleal lamella so that the latter never extends to the free border of the mesentery.

In Figs. 10 to 14, Pls. II. and III., are shown a number of sections through the same laceration embryo at different levels. In Fig. 10, which passes close to the oral disc, only four complete mesenteries are present while a member of the third pair has made its appearance on one side of the longitudinal axis of the stomodeum.

In Fig. 11, a section taken through the embryo near the base of the stomodeum, seven mesenteries are present. The same four as noted in the previous section are complete while both those of the third pair are present and one of the fourth pair is seen as a slight prominence of the endoderm containing a very thin mesogleal fold. In Fig. 12 only six mesenteries are present, but it seems that one of the third pair is lacking instead of the single one of the fourth pair which was seen in the previous section.

The first pair have already acquired their mesenteric filaments, which at this stage extend only a short distance below the stomodeum.

Farther down, at the level of the section shown in Fig. 13, only four new mesenteries are present, while seven of those which came over in the laceration piece from the parent are seen. This last-mentioned section was taken about one third of the distance from the oral disc to the base of the embryo, and in this series the newly formed mesenteries are found in only four of the following sections.

In the sections shown in Fig. 14, all of the mesenteries present are those which were in the laceration piece at the time when it was separated from the parent. Fig. 14, although taken at some distance from the base shows, in comparison with the previous sections, that the basal portion of the embryo is of considerably greater diameter than the more distal portions.

A comparison of the three last-mentioned figures illustrates one phase of the redistribution of materials in the growth of a laceration embryo. In Fig. 13, that side where the new mesenteries are being formed is very thin-walled, the mesoglea having become reduced to a thin sheet throughout that half of the body. In the remaining half, on the other hand, the tissue relations of an adult *Aiptasia* are still maintained. In the sections through the more proximal part of the body the adult tissue relations are apparently undisturbed, at least morphologically. It will be seen, however, that in this region there has been coincident with the elevation of the oral end of the embryo a drawing together of the basal portion so that it is now nearly circular in outline. In Fig. 15 it may be seen that the old mesenteries which formerly occupied only one side of the fragment—at the time of its separation from the parent—have now come to be distributed so that they now arise from about four fifths of the circumference.

In sections through an older specimen, as shown in the series of figures from Fig. 15 to Fig. 18, the second set of mesenteries has made its appearance about the inner surface of the oral disc, and eighteen of them have reached the stomodeum. Farther down along the stomodeum (Fig. 17), the twelve primary mesenteries are complete while only one other pair has made sufficient downward growth to appear in such a section.

In Fig. 18, where eleven of the twelve primary mesenteries are present, none of those which came over in the laceration piece from the parent animal are in evidence. Eight of the new mesenteries have acquired their filaments, but only those on the first pair of mesenteries are trilobed.

As a basis for the comparison of this series with the previous one attention may be called to the appearance of the mesoglea in Fig. 18. At a corresponding height on the column of the individual shown in the former series (Fig. 14), none of the newly formed mesenteries were present, while in Fig. 13, where only four of them had appeared, that side of the column on which they were situated showed all of the characteristics of newly formed tissue.

This shows beyond question that in the older specimen the new mesenteries have extended down to a part of the body where there were formerly some of the older mesenteries which by this time have been entirely resorbed. It will be noticed that in this part of the body the tissue readjustment has proceeded until the relation between the layers characteristic for the adult has been reached.

Carlgren, 1904, in discussing a series of sections through an embryo in which eight of the new mesenteries had reached the stomodeum figures two sections and makes statements from which I can infer only that he understands, either that some of the old mesenteries are brought into the permanent systen of mesenteries of the laceration embryo, or that some of the new mesenteries arise at a level below the lower end of the stomodeum. Either of these conclusions is at variance with the results which I have obtained in the study of the four species of actinians on which the present paper is based.

Carlgren's figures and the statements directly relating to them are as follows:

Ein querschnitt in der Schlundrohrsregion (Fig. 4, Taf. IX.) zeigt ein birädial Anordnung der vollständigen Mesenterien mit den beiden Enden des spaltförmigen Schlundrohrs sind zwei Richtungsmesenterienpaare vereinigt. An jede Seite derselben liegen zwei vollständige Mesenterien (*a*) ich nenne sie später kürzlich seitliche Mesenterien—die ihre Längsmuskelen gegen einander kehren. In allem sind also 8 Mesenterien vollständig.

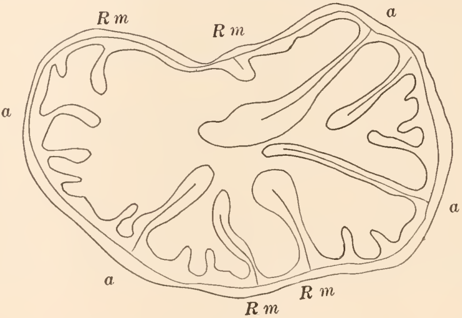

FIG. 1. (Carlgren's Fig. 4, Taf. IX.)

(Wenn ich unter von einer birädialen Anordnung sprache, sind die vollstandigen Mesenterien wie hier grupiert.) Zwischen den Seitlichen Mesenterien an jeder Seite sind verscheidene, unvollständige Mesenterien entstanden, die in dem proximal körpertheilen (Fig. 7, Taf. IX.), ziemlich staak sind; in den distalen an der einen Seite verschwinden, an der anderen nur unbedeutend entwickelt sind oder ganz fehlend (Fig. 4, Taf. IX.). Die mit (*b*) bezeichneten Mesenterien bilden mit ihren Partneren an je ein Mesenterienpaar mit zugewandten Langsmuskeln. Die Mesenterien des Stückchens sind also nach der Sechszahlangeordenet, obgleich von dem ersten Cyklus vier Mesenterien unvollständig sind. Die zwischen den Mesenterien (*b*), liegenden

Mesenterien gehören einem zweiten Cyklus an. An jeder Seite der Richtungsmesenterien findet man auch unvollständige Mesenterien eines zweiten Cyklus. Auch einige Mesenterien der dritten Ordnung sind angedeutet (Fig. 7, Taf. IX.). An der Bases sind die mit (*a*) bezeichneten Mesenterien am Stärksten, die Mesenterien (*a*) an der rechten Seite der Sagittalachse laufen fast mit den entsprechenden Mesenterien an der anderen zusammen (Fig. 7, Taf. IX.).

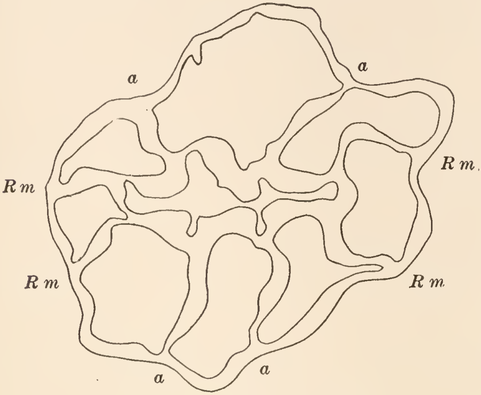

FIG. 2. (Carlgren's Fig. 7, Taf. IX.)

His Fig. 4, Taf. IX., my text Fig. 2, shows a condition of the mesenteries which is common to all laceration embryos in a corresponding stage of development. His Fig. 7, Taf. IX., text Fig. 3, shows twenty-five mesenteries, eight of which he considers to be of the first cycle (mesenteries marked *a*) while of the remaining seventeen six are according to his statement of the second cycle (marked *b*) and the remainder of a third cycle. It will be noticed that several of the mesenteries in this figure—one pair of those marked *Rm* and the longest ones at one end of the figure—are very broad in section with a thick mass of endoderm extending far beyond the mesoglea. None of the entire number of mesenteries have any trace of mesenteric filaments, nor by their structure, in so far as it can be made out from the original figures, is there any indication that they contain actively growing tissue as is always found in the new mesenteries in the forms that I have studied. Comparing these two figures with those of the series shown in my Figs. 10 to 14, Pls. II. and III., it seems very evident that all of the mesenteries shown in Carlgren's Fig. 7, Taf. IX., are old ones which have come over in the fragment from the parent individual and which will never come to be a part of the permanent system of mesenteries of the actinian arising from the laceration embryo. In the younger embryo shown in my series of figures just mentioned the first formed pair of mesenteries has already acquired their mesenteric filaments, which are as yet, of course, in an undifferentiated condition; but nevertheless clearly distinguishable as mesenteric filaments.

In the embryo from which my series of Figs. 16 to 18 was taken, which has at the level of the stomodeum only four more mesenteries than that from which Carlgren's figures under discussion were taken, eight of the new mesenteries, at the level at which Fig. 18 was taken, are provided with filaments of which those on the first pair of mesenteries are already trilobed, while several of the others show the first steps in the process of differentiation by which the trilobed condition is reached.

(*c*) *The Development of the Mesenteric Filaments.*

The question of the origin of the mesenteric filaments among the anthozoa has been discussed to considerable length by most workers on the development of these forms. With the exception of E. B. Wilson, 1884, all of the previous investigators have confined themselves to a consideration of the embryonic development. The above-named investigatór worked with the polyps arising by budding in several alcyonarians, but not with embryos of the same forms.

E. B. Wilson (*loc. cit.*) reached the conclusion that in the Alcyonaria the dorsal directive filaments arise as downgrowths of the ectoderm of the stomodeum, while all the other filaments arise through the differentiation of the endoderm at the free border of the mesentery. He would homologize the dorsal filaments of the Alcyonaria with the lateral ciliated bands (flimmerstriefen) of the trilobed actinian filament. The filaments of the remaining mesenteries he would homologize with the glandular streak (Nesseldrüsenstreif) of the trilobed filament. Thus, according to his interpretation, the actinian filament is a double structure.

H. V. Wilson (*loc. cit.*) working on the coral *Manicina*, where the filament consists of a single lobe, reached the conclusion that this type of filament was comparable to the trilobed actinian filament and is entirely of ectodermic origin.

McMurrich, 1891, describes a double origin for the trilobed filaments of *Rhodactis* and *Aulactinia;* after the manner suggested by E. B. Wilson.

Duerden, 1899, working on *Lebrunia*, where the mesenteries become developed before the stomodeum becomes hollowed out, concluded that in this case part, at least, of the filaments are of endodermic origin since he finds stages where filaments are present on some of the mesenteries which as yet have no direct connection with the stomodeum.

Appellöf (*loc. cit.*) concludes that the mesenteric filaments are developed entirely from the ectodermal downgrowth from the lining of the stomodeum. He mentions having observed a number of instances where the ectodermal layer had become very much attenuated just below the level of the stomodeum, and indeed in some instances the continuity of this layer had been broken. He argues from this fact that some of the instances observed by previous workers who, on the basis of a lack of continuity between the mesenteric filament and the lining of the stomodeum, maintained the endodermic origin of the filaments might be simply cases where the continuity of the tissue had been broken and in no way fundamentally different from the ordinary course of events as he has described them. He also makes the suggestion that the method of development described by E. B. Wilson for the alcyonarian filaments may be confined to those individuals which arise as buds and not a true explanation of what takes place during embryonic development, which he tacitly assumes would be similar to plans followed in other Anthozoan forms.

In all descriptions of the formation of the mesenteries, and particularly of the mesenteric filaments, except that of Duerden (*loc. cit.*) for *Lebrunia*, it is recorded that the filaments appear first on the upper part of the mesenteries at the level of the free border of the stomodeum, and usually there can be observed a direct continuity between the ectodermal lining of the stomodeum and the filaments from the first appearance of the latter.

The mesenteries acquire their filaments in the same order as that in which they arose, so that the dorso-laterals would first show these structures. In Fig. 12, Pl. II., this pair of mesenteries alone shows the beginning of the filaments. Farther up in this series of sections the tissue forming the filaments is directly continued into the lining of the stomodeum. In Fig. 9, Pl. II., a longitudinal section through a young laceration embryo, the filament extends for a short distance down along the free border of the mesentery, and here there is no histological differentiation to mark the lower limits of the stomodeum. Its actual limit can, however, be determined by a comparison with the other side of the figure where the section passed between two mesenteries so that the border of the stomodeum hangs free in the gastrocœl. H. V. Wilson (*loc. cit.*) has pointed out that in *Manicina* the filaments of all the first twelve mesenteries, save the first pair, arise from a fold of the ectoderm at the free border of the stomodeum which bends up and outward into the gastrocœl pointing toward the oral disc. In this manner the filament begins to grow down the mesentery before the edge of the latter has in its upper part come into contact with the stomodeum.

In the development of laceration pieces of *Aiptasia* there is the same folding backward of the wall of the stomodeum at an early stage in the development (Fig. 8), so that the course of the development of the filaments in this form follows that already described for *Manicina*.

In an older specimen, Fig. 18, Pl. II., the filaments of the first pair of mesenteries have assumed the trilobed form characteristic of the filaments of adult *Aiptasias*, while the remainder of the mesenteries have at this level either simple filaments or none at all. It is thus apparent that the change in the structure of the filaments follows the same order as does the appearance of the filaments themselves.

In this transition from the simple to the tri-lobed structure there is in these forms no indication of the origin of the ciliated bands as an acquisition of new or different tissues, as McMurrich has suggested in arguing for a dual origin for the parts of the complex filament, but, on the other hand, the lateral lobes of the complex filament arise by a very slight differentiation of the cells already present in the filament from the time of its origin from the stomodeal ectoderm. In Fig. 17, Pl. III., the filaments of the dorsal directive, the ventro-lateral and the ventral directive mesenteries all show in their centers the beginning of the growth which will give to the mesogleal layer its characteristic branched appearance. Each of these branches will become the center and support, of one lobe of the adult trefoil filament. In the dorsal directive mesenteries especially, it may be noticed that the tissues of the filament have extended out around the edges of the mesentery so that the filament is nearly circular in outline. Originally the extension of the stomodeal ectoderm—first appearance of the filament—was in the form of a flat sheet (Fig. 12, Pl. II.). The separation of this sheet of tissue into the three lobes of the adult filament apparently takes place through an unequal growth from three points in the nearly circular filament, as shown in Fig. 18, Pl. III.

As may be seen by comparing Fig. 9 and Fig. 12, Pl. II., with the fully formed filament (*e. g.*, the filaments of the dorso-lateral mesenteries in Fig. 17, Pl. III.) the histological changes which have taken place in the transformation of the "embryonic" tissue of the stomodeal ectoderm to those of the tri-lobed filament have been in the nature of a separation of the cellular elements of the original tissue so that one type of cells (ciliated) is centralized in the lateral lobes; while the other types (gland and nettle cells) are concentrated in the median lobe. The apparent change is greatest in the case of the median lobe, since the undifferentiated stomodeal ectoderm is at the time of the first appearance of the filaments poor in gland cells and nematocysts. This last-mentioned fact is also true of the activity growing ectoderm of the new part of the column wall and oral disc, and in all parts of the body there is a marked increase in the actual as well as in the proportionate number of both these types of cellular elements after the normal body proportions have been established.

(*d*) *Histological Changes During Development by Laceration.*

In considering the histological changes undergone by the tissues during development by pedal laceration those changes which take place in the basal portions of the parent individual before the fragment is torn off may be first mentioned. Andres (*loc. cit.*) mentions that in *Aiptasia lacerata* the rim of the basal portion of the animal becomes very opaque, and that in section there seems to be an unusual number of gland cells in this region. The endoderm becomes very much thickened in this region so that when the fragment becomes torn off there is only a small cavity left in its interior (Fig. 7, Pl. II.). As may be seen in this section, the thickening of the endoderm is very much greater on the upper side of the fragment, *i. e.*, that which has come from the original column well.

In the period immediately following upon the separation of the fragment from the parent, there is a rapid change in the character of the tissues about the orifice. All of the newly forming tissue becomes very light in color due to the comparative paucity of the zoöxanthellæ in the endoderm of the active area. The mesoglea becomes comparatively thin in this region, but seems always to arise as a direct prolongation of that already present and not as a new secretion between the ectoderm and endoderm of the new parts.

In the cellular layers the changes are more profound. In the ectoderm (Fig. 19, Pl. IV.), a section through a young tentacle bud, the boundaries of all of the cells except the nematocysts and gland cells becomes lost so that the whole layer constitutes a syncytium. The cytoplasm is uniformly granular throughout. The nuclei are scattered through the cytoplasm, but still indicate by their positions the fact that only definite cell walls are wanting in order that the usual cell structure should be apparent. At the internal boundary of the entoderm there is distinguishable a layer of very fine muscle fibers. When compared with the size of the nuclei and nematocyst cells, these fibers are seen to be several times less than the diameter of normal muscle fibers of this species of actinian. Since in the fully developed *Aiptasia* muscle fibers exist both as processes from epithelio-muscle cells and as nearly the entire component of "muscle cells" in which the cytoplasmic cell body is reduced to a small mass containing the nucleus, it is impossible in this case to determine whether we have to do with fibers of the first or of the second of the two above-mentioned kinds. In other words the fibers in question may be in the process of becoming differentiated from epithelial cells, or they may be arising through the division of previously existing muscle cells of the second type. At this stage there is no indication of interstitial cells or of developing gland cells in the ectoderm.

In the endodermal layer of the same section there is exhibited a similar condition of the tissue: Cell walls are entirely wanting; the nuclei, however, by their position indicate a potential cellular arrangement of the cytoplasm. A particularly striking feature as regards this layer is brought out by a comparison of the figure under discussion and Fig. 7, Pl. II. In the last-mentioned figure the endoderm, throughout most of its extent, is considerably thicker than the ectoderm, while in the new tissue the former is generally so thin that the single zoöxanthellæ are greater in diameter than the layer of cytoplasm making up the endoderm and consequently they cause a prominence extending into the cavity of the tentacle wherever they are situated. In some few instances the zoöxanthellæ are placed one above the other, and n such cases there is either a generally thickened area of the cytoplasm or else a cylindrical prominence, as is seen on one side of the figure. All cellular differentiation is entirely lacking in the endoderm. At no place about the circumference of this layer is there any indication of the layer of endodermal (circular) muscles, which are in normal *Aiptasia* tissues, when seen in transverse sections of the animal, much more prominent than the ectodermal muscles.

At the base of the fragment where the rearrangement of the materials is proceeding at a relatively slow rate there seems never to be a breaking down of the tissues comparable to that just described. The mesogleal layer becomes thinned out progressively so that at first it is of its usual thickness on one side of the fragment—that side originally opposite the point where the fragment was torn off from the parent animal—while on the opposite, more actively growing, side it has become relatively thin (Fig. 13, Pl. III.). The cellular layers retain practically their original characteristic appearance, although, as is shown in Fig. 15, Pl. III., the ectoderm on the side where the new growth is the most rapid, *i. e.*, where there are no old mesenteries in the figure, is much lower than on the opposite side.

When in the later development the cellular elements of the ectoderm and endoderm become delimited by the appearance of cell walls it is noticeable that all of the cells show at first an embryonic character in that they are relatively wide in proportion to their height (compare Figs. 10 and 11 with the old part of the tissue in Fig. 14). In the ectoderm of the column wall in Figs. 10 and 11, and more especially in the lining of the stomodeum of this individual, there is a very marked paucity of the nematocysts and gland cells, so that it is apparent that there has not been an increase in these cellular elements to keep pace with the increase in the epithelial cells. In the older embryo from which the series of figures from 15 to 18 was taken the nematocysts and gland cells have reached nearly their normal number and distribution throughout the newly formed tissues.

In the sections shown in Figs. 10 and 11 the endoderm cells are in general broader than the ectoderm cells and not generally of greater height. In the older tissue (Fig. 18) there are no apparent cell boundaries in the endoderm and the zoöxanthellæ are much more numerous.

SUMMARY.

1. The change in the form of a laceration piece, leading up to the acquisition of the typical actinian shape, takes place through the upgrowth of the tissue about the orifice where it was torn off from the parent.

2. The permanent mesenteries arise as new growths in the undifferentiated tissues of the oral end of the laceration piece.

3. The first twelve mesenteries do not appear in the sequence followed by those in egg embryos.

4. As development goes on the old mesenteries—those brought over from the parent when the fragment was torn off—become restricted to a proportionately shorter and shorter part of the base of the young actinian until they are finally entirely resorbed.

5. The mesenteric filaments are formed, just as in egg embryos, from a downgrowth of the ectodermal lining of the stomodeum. Their trilobed condition arises through the differentiation of this tissue.

6. The tissues of the most actively growing part of a laceration piece become very thin; the ectoderm and the entoderm lose all apparent cell boundaries; the mesoglea arises as a direct continuation of that present in the older tissues.

7. The newly formed tissues contain a very small number of gland cells and nematocysts. These two types of cells are developed to the usual number after the tissue relations have bebecome stable.

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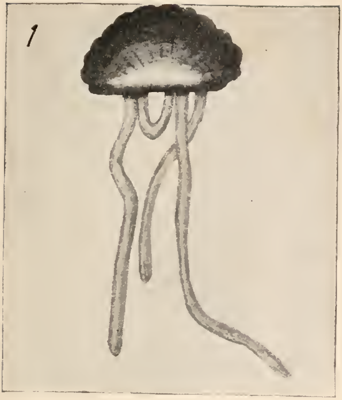
Explanation of Plates.

All the figures were drawn from specimens of *A. pallida;* those of the entire laceration pieces from specimens grown in the laboratory; the sections from specimens obtained at Beaufort, N. C.

The ectoderm is stippled in all the figures, the endoderm is distinguished by the presence of the zoöxanthellæ, the mesoglea has been represented by either a single line in the actively growing parts of the embryo or by a clear space between the cellular layers in the older tissues.

Plate I.

The figures on this plate represent a series of stages in the development of a laceration piece, from the time of its separation from the parent until it has assumed the form of a typical young actinian.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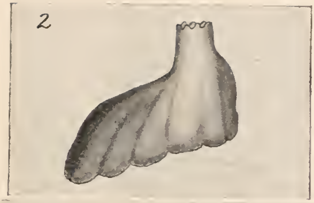
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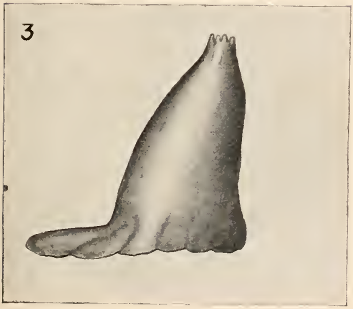
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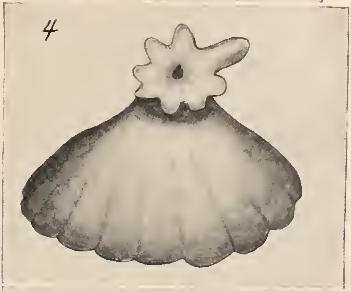
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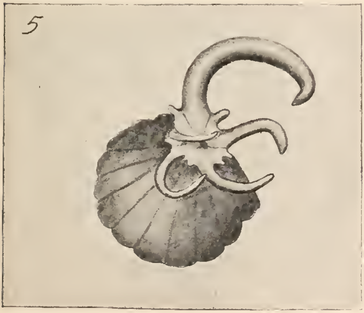
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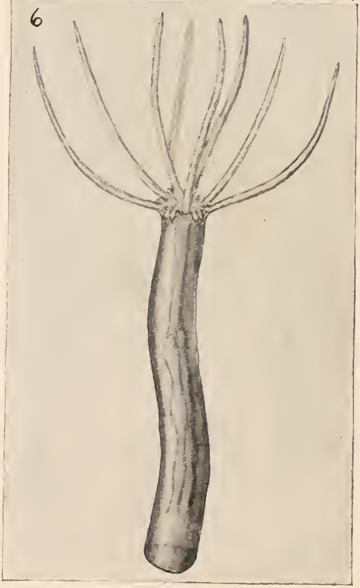
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PLATE II.

FIG. 7. A section through a laceration piece soon after its separation from the parent.

FIG. 8. Part of a vertical section through a young bud to show the formation of the stomodeum.

FIG. 9. Part of a longitudinal section through a young laceration embryo, such as is shown in Fig. 4, Plate I., to show the downgrowth of the ectodermal lining of the stomodeum to form the mesenteric filaments.

FIGS. 10, 11, 12 (with FIGS. 13 and 14, PLATE III.). A series of sections at different levels taken through the same embryo to show the relation of the new to the old mesenteries. (The orientation is the same in all the figures.)

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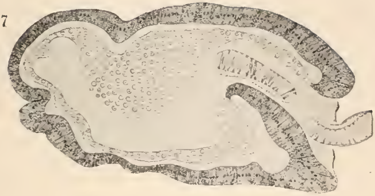

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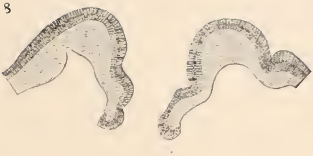

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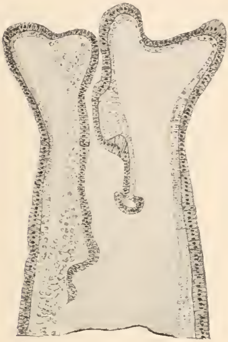

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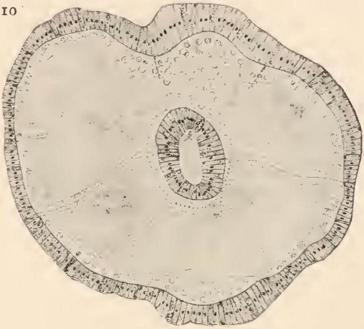

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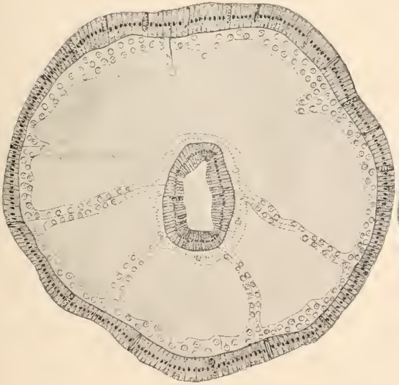

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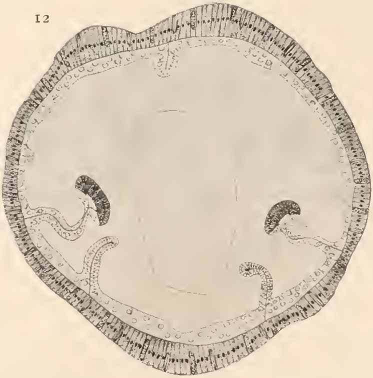

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Plate III.

Figs. 13 and 14 are part of the series described in the last paragraph. They complete the series which began with Fig. 10.

Figs. 15, 16, 17 and 18. A series of sections, at different levels, through an older embryo in which all of the twelve primary mesenteries have reached the stomodeum.

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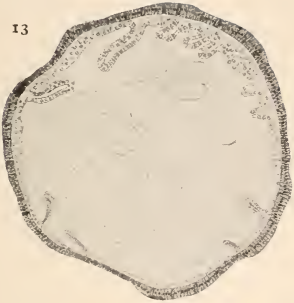

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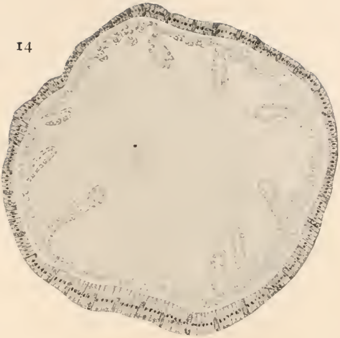

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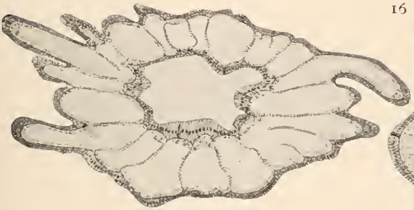

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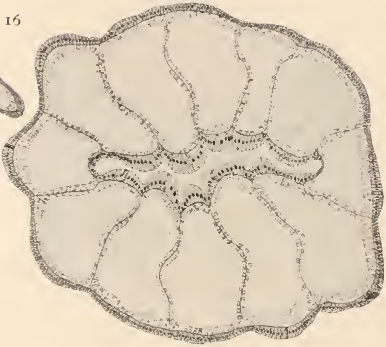

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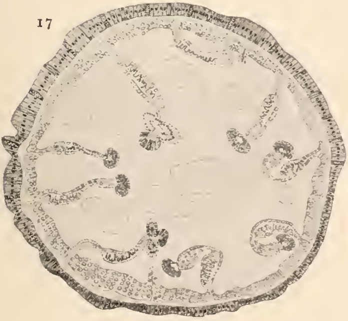

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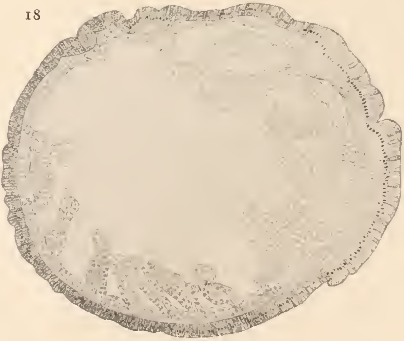

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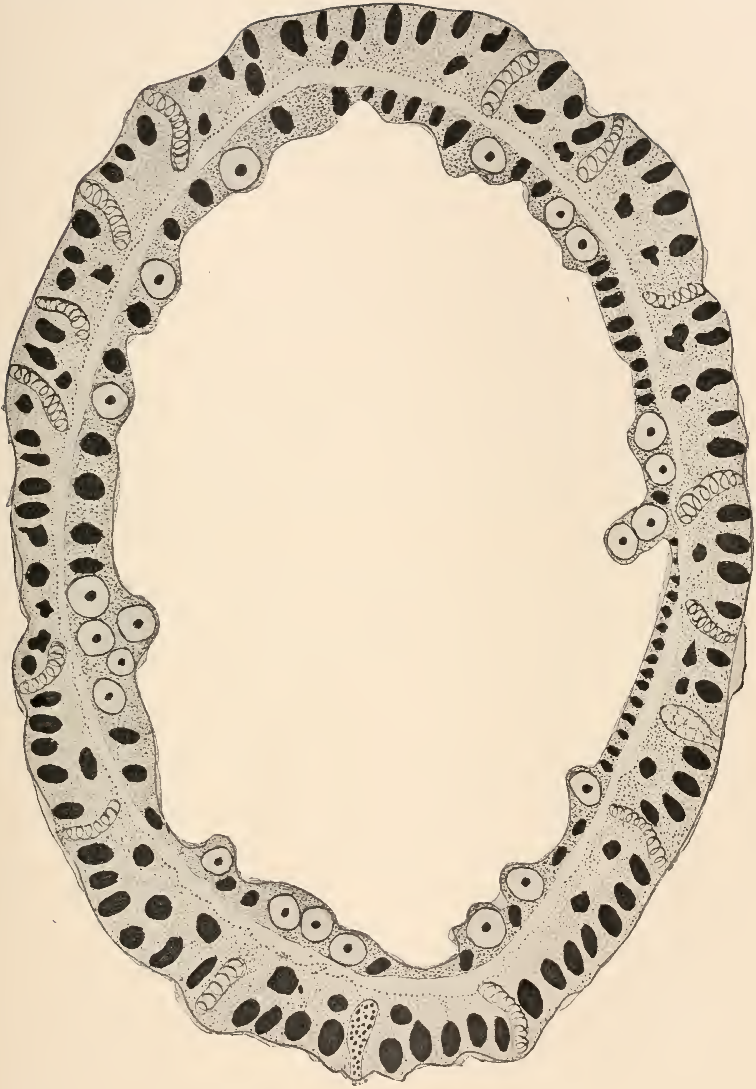
PLATE IV.

FIG. 19. A section through a very young tentacle bud to show the characteristics of the tissues in an actively growing region of the developing laceration embryo.



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FURTHER STUDIES ON HETEROCHROMOSOMES IN MOSQUITOES.

N. M. STEVENS.

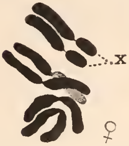
In my study on the chromosomes in the germ cells of *Culex*, published in March, 1910 ('10), there were three figures (11, 20 and 21) which differed from all others for *Culex*, in showing an unequal pair of chromosomes in the spermatogonia (Fig. 11), and a condensed pair in the growth stage of the spermatocytes (Figs. 20 and 21). At the time when the proof of that paper was corrected, it was suspected that the three figures referred to belonged to another species, probably *Anopheles punctipennis*, a few larvæ of *Anopheles* having, it was supposed, been collected in the net with *Culex*, and having escaped detection in both larva and pupa stage.

In October of this year (1910) an abundant supply of *Anopheles* larvæ was secured and a satisfactory study of both male and female germ cells made, showing without question that in the male *Anopheles punctipennis* there is present an unequal pair of heterochromosomes, attached to one of the equal pairs, but otherwise exactly comparable to the unequal pair described by the author for nine species of Muscidæ ('08). A careful reëxamination of the germ cells of *Culex pipiens*, and a study of *Culex tarsalis* and *Theobaldia incidens* in California during the summer, revealed no such heterochromosomes in those species. A description of the chromosomes in the germ cells of *Anopheles punctipennis* and *Theobaldia incidens* will be given in the following pages.

Methods.—The germ glands of both *Anopheles* and *Theobaldia* were studied both in acetocarmine preparations and in sections of material fixed in Flemming and stained with iron-hæmatoxylin or thionin. The latter stain gave very satisfactory results with this material, and saved much time in preparing the sections for study.

Anopheles punctipennis.

Very clear oögonial plates were found in several ovaries, and two of these are shown in Figs. 1 and 2. There are two equal pairs of chromosomes very similar to those found in other Diptera, and a third pair which is clearly made up of a longer and a shorter pair. Corresponding figures of spermatogonial plates are shown in Figs. 3 and 4. Here the composite pair consists of a longer equal pair, as in the oögonia, united with a smaller unequal pair, the larger member of which is of the same relative size as the members of the corresponding small pair in the oögonial plates. All of the spermatogonial plates in this *Anopheles* material were of this type. In *Culex*, on the other hand, no such inequality or composite condition of one pair of chromosomes could be detected. Figs. 5 (♀) and 6 (♂) are new figures of the oögonial and spermatogonial chromosomes of *Culex pipiens*, and those of *Culex tarsalis* are of the same general character.



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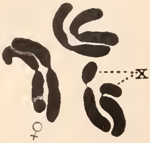

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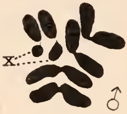

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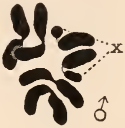

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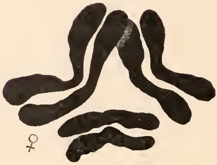

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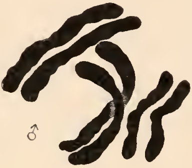

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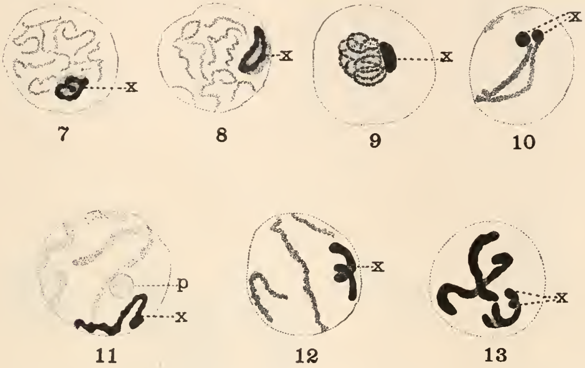
FIGS. 1 and 2. *Anopheles punctipennis*, chromosomes of the oögonia. Mag. 2,000.

FIGS. 3 and 4. *Anopheles punctipinnis*, chromosomes of the spermatogonia. Same mag.

FIG. 5. *Culex pipiens*, chromosomes of an oögonium. Same mag.

FIG. 6. *Culex pipiens*, chromosomes of a spermatogonium. Same mag.

In resting spermatogonia of *Anopheles* (Figs. 7 and 8) a condensed mass of chromatin varying in form is found associated with a large plasmosome near the nuclear membrane. In *Culex* there is nothing of the kind—only a large central plasmosome surrounded by an even spireme. In *Anopheles* there is a very conspicuous contraction or synizesis stage (Fig. 9) in which the heterochromosome appears as a deeply staining mass of chromatin resting against a ball of paler chromatin threads. The corresponding stage in *Culex* ('10, Figs. 12 and 13) showed a ball of chromatin thread wound about a plasmosome. In the later growth stages of the spermatocytes of *Anopheles*, the heterochromosome pair assumes a great diversity of forms. Occasionally, especially in early stages, the unequal ends of the pair only are fully condensed as shown in Fig. 10, which is a tangential section of the nucleus. Fig. 11 shows a late spireme stage with heterochromosome (x), plasmosome (p) and parts of a pale spireme. Fig. 12 is a late growth stage taken from a cyst where the cells were mostly in metaphase of the first spermatocyte mitosis; here the heterochromosome pair shows nothing of its composite



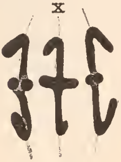
FIGS. 7 and 8. *Anopheles punctipennis*, resting spermatogonia. X = the heterochromosome.

FIG. 9. Synizesis stage.

FIGS. 10–12. Growth stages.

FIG. 13. Prophase.

nature, and the members of the pair are in about the position which they assume in the spindle. Throughout the growth stage the composite pair of chromosomes is found, more or less condensed, but always distinguishable from the remainder of the chromatin—against the nuclear membrane, twisted or coiled or knotted into such shapes that it is usually impossible to distinguish the equal and unequal pairs of which it is composed.



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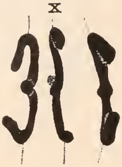

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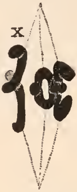

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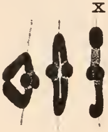

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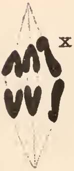

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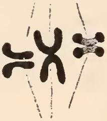

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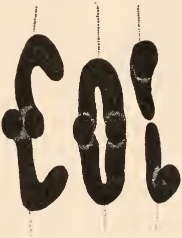

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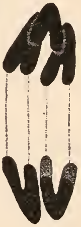

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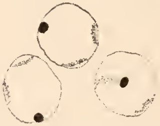

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FIGS. 14–17. *Anopheles punctipennis*, first spermatocyte metaphase. *X* = the unequal pair.

FIG. 18. Anaphase.

FIGS. 19 and 20. *Culex pipiens*, first spermatocyte metaphase and anaphase.

FIG. 21. *Anopheles punctipennis*, second spermatocyte metaphase.

FIG. 22. Spermatid nuclei. All figures same magnification—2,000.

Prophase stages are evidently much briefer than in *Culex*, for it was difficult to find any clear figures—Fig. 13 shows the composite heterochromosome pair (x) and the other two chromosomes not yet separated into their parallel components.

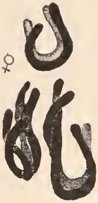
The character of the chromosome pairs as they appear in the first maturation mitosis is well shown in Figs. 14–17, where the chromosomes are represented in one plane for the sake of clearness. In each figure x is the composite pair. The spindle fibers appear to be attached at the meeting point of the smaller and larger components, the smaller, unequal pair forming the outer ends of the figure and appearing as a conspicuous hook at one end and a rounded knob at the other. No distinct separation of the members of the composite pair, such as is always seen in the spermatogonia (Figs. 3 and 4) was ever observed in the spermatocyte metaphase. The other two chromosomes frequently assume the form of rings in the early metaphase (Figs. 16 and 17) and in the anaphase (Fig. 18) they separate as V's with equal arms, while the heterochromosomes form a pair of hooks with equal long arms and unequal short arms (Fig. 18).

Fig. 19 shows the chromosomes of a first spermatocyte metaphase of *Culex pipiens* and Fig. 20 an anaphase. The chromosomes correspond very closely in form and relative size to those of *Anopheles* with the exception that all three pairs are equal. Both cells and chromosomes are much larger in *Culex* than in *Anopheles*.

In the second spermatocytes the chromosomes are already divided when they come into the spindle and are usually so tangled as to defy any attempt to distinguish the two classes or make clear drawings. In Fig. 21 we have an unusually clear metaphase. An aggregation of chromatin, apparently the heterochromosome, is found in the spermatids (Fig. 22), lying against the nuclear membrane, and in some cases the dimorphism can be distinguished.

Theobaldia incidens.

Theobaldia incidens is one of the most common mosquitoes in the Santa Clara Valley, California. The larvæ were found in abundance in a bucket of water which had been allowed to stand partly covered for two or three weeks. A few bread crumbs thrown into the glass jars in which the larvæ were kept under observation, enabled them to grow rapidly and transform into fine large pupæ whose germ glands yielded all desired stages, from dividing oögonia and spermatogonia to ripe spermatozoa.



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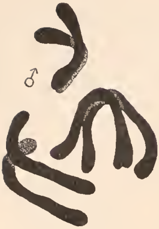

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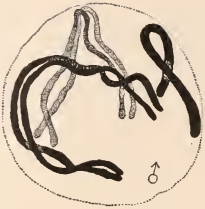

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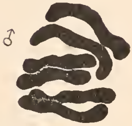

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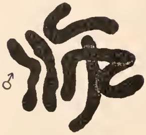

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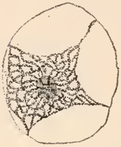

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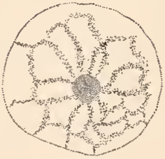

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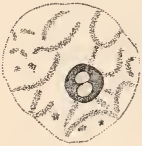

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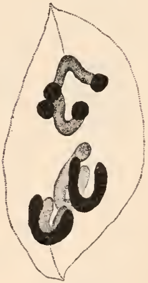

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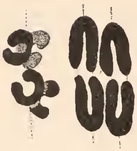

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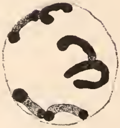

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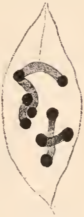

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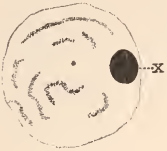
FIG. 23. *Theobaldia incidens*, oögonial prophase.
FIGS. 24–26. Chromosomes of the spermatogonia.
FIG. 27. Spermatogonial prophase.
FIG. 28. Synizesis stage.
FIGS. 29 and 30. First spermatocyte growth stages.
FIGS. 31 and 32. First spermatocyte prophase and metaphase.
FIGS. 33 and 34. Second spermatocyte prophase.

During the summer only acetocarmine preparations were used and the material resembled *Culex* so closely that only a few figures were made. Other figures were taken from sections of ovaries and testes fixed in Flemming and stained with thionin. An oögonial prophase is shown in Fig. 23, and the chromosomes from three spermatogonia in Figs. 24, 25 and 26. Fig. 27 is the nucleus of a spermatogonium in prophase. In no one of these figures is there any indication of inequality in the members of any pair of chromosomes. Neither in resting spermatogonia nor in the growth stages of the spermatocytes could anything resembling the heterochromosomes of *Anopheles* be found. In all of these stages a central plasmosome is surrounded by a spireme as in *Culex*. Figs. 28 to 30 show a contraction or synizesis stage, and two later growth stages. As in *Culex* the plasmosome stains a deeper blue in late growth stages than in the spermatogonia and synizesis stages. Fig. 31 is a characteristic prophase of the first spermatocyte mitosis. In the metaphase two of the chromosme pairs nearly always appear in the form of rings and separate as V's; the third pair is usually separated and its univalent components already divided longitudinally, when the rings are just dividing (Fig. 32). The second spermatocyte prophases (Figs. 33 and 34), metaphases and anaphases resemble closely those of *Culex*.

The testes of *Theobaldia* differed from those of *Culex* in having much larger spermatogonia and in lacking a prolonged prophase of the first spermatocyte mitosis. The first maturation division also shows characteristic differences in the behavior of the chromosome pairs in metakinesis (compare Figs. 19 and 32). Otherwise both ovaries and testes showed similar conditions in the two species, *Culex pipiens* and *Theobaldia incidens; i. e.*, three equal pairs of chromosomes, no one of which behaves in any respect like the heterochromosomes of other insects, including *Anopheles punctipennis*.

Anopheles sp.?

On March 30 and again on May 31, a few pupæ were obtained from the same pond where I obtained my material in 1909. All of the males in these collections had six chromosomes in the spermatocytes and a distinct heterochromosome in the growth stages. The pupæ were thought to be *Anopheles* but no larvæ or adults were secured for identification. It was supposed that the few specimens obtained must have wintered over, probably in



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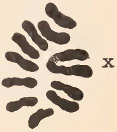

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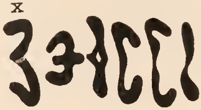

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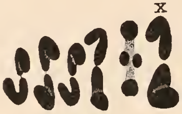

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FIG. 35. *Anopheles* sp.? First spermatocyte growth stage. X = the heterochromosome.

FIG. 36. First spermatocyte prophase.

FIGS. 37 and 38. Chromosomes of the first spermatocyte metaphase. Mag. 2,000 in all figures.

the larval stage. The net was used in the pond many times during March, April and May, but no mosquito larvæ or pupæ secured except on the two dates given above.

Fig. 35 shows the heterochromosome in the nucleus of a growth stage, Fig. 36 a prophase of the first maturation mitosis, and Figs. 37 and 38 the six chromosomes from two first spermatocyte spindles. The largest pair is slightly unequal but not composite as in *Anopheles punctipennis*.

DISCUSSION.

As we have already seen, the various species of Culicidæ, thus far studied, present peculiarities not hitherto encountered in the study of heterochromosomes in the various groups of insects. In *Anisoloba maritima* (Randolph, '08) and several species of Lepidoptera (Stevens, '06; Dederer, '07; Cook, '10) there is an equal pair of heterochromosomes distinguishable as such by their condensed condition in the growth stages of the first spermatocytes. In *Drosophila ampelophila* the unequal pair of heterochromosomes of the maturation mitoses is not condensed during the growth stages (Stevens, '08). In *Metapodius terminalis* (Wilson, '09) we find either an unpaired heterochromosome or an unequal pair of idiochromosomes, and a varying number of supernumeraries within the same species. In no case before have undoubted heterochromosomes, following the usual rule as to sex distribution, been found in one or more genera or species and entirely absent in nearly related species of the same family. In *Anopheles punctipennis* the heterochromosomes can be traced from the youngest spermatogonia observed, through the two generations of spermatocytes to the spermatids and even to a stage in which the spermatids are transforming into spermatozoa. The unequal pair in the male is represented by an equal pair in the female, and as in other similar cases, it is evident that an egg fertilized by a spermatozoön containing the smaller heterochromosome must produce a male, while an egg fertilized by a sperm containing the larger heterochromosome will develop into a female.

Now in *Culex* and *Theobaldia* all of the chromosomes behave alike so far as condensation is concerned, and there is neither an unpaired heterochromosome nor an unequal pair to be detected in any stage. In other words there is no visible differentiation of one pair of chromosomes which can be associated with the determination of sex. There seems to be absolutely no reason for supposing that the mechanism by which sex is determined is not identical in these three species of mosquitoes; and the inevitable conclusion would seem to be either that the heterochromosomes of the male are not a necessary factor in sex determination, or that the sex-determining chromosomes are not necessarily differentiated as heterochromosomes.

I have kept no record of the sex of the pupæ dissected, but should say that the numbers of males and females were about equal for all of the species studied. In a large collection of *Theobaldia* larvæ which, judging by their size, might have all come from one laying, most of the males pupated a day or two sooner than the females. All of the pupæ were taken out and dissected each day. The first day nearly all were males and the last day nearly all females. An average for the four or five days, I feel sure, must have given nearly equal numbers.

Wilson's recent hypothesis ('09) that in some way two X chromosomes determine the female sex, and one X chromosome or an X and a Y chromosome determine the male sex would apply perfectly to *Anopheles.*

$$\text{Egg } X + \text{sp. } X = ♀,$$
$$\text{Egg } X + \text{sp. } Y = ♂.$$

But what shall we say of *Culex* and *Theobaldia* where there is no differentiation of X and Y chromosomes? In forms which have an equal pair of heterochromosomes, showing the same general characteristics as the unpaired heterochromosomes and the idiochromosomes, one naturally infers that the members of the equal pair in the male have X and Y values respectively, but shall we go further and argue that likewise in *Culex* and *Theobaldia* the mature eggs all have an X chromosome and the spermatozoa are dimorphic, one half having an X chromosome and the other half a Y chromosome, although nothing of the kind can be traced? If we make this assumption, then *Culex* and *Theobaldia* may stand at one end of a series, as examples of absence of heterochromosomes associated with sex, while forms with an unpaired heterochromosome come at the other end of the series, and all the varieties of paired heterochromosomes between, the mechanism of sex determination being the same in all, but evident to the eye only when differential heterochromosomes are present.

The case of the aphids and phylloxerans has been the strongest argument for the hypothesis that two X chromosomes give a female and one X or XY a male, since the rejection of one X chromosome from a parthenogenetic egg is followed by the development of a male, but in *Culex* and *Theobaldia* it is evident that sex determination is not dependent on the presence of X and Y chromosomes, although in *Anopheles* these chromosomes are at least closely correlated with sex-determination.

At present, the all-important questions seem to me to be: What is the meaning of the differentiation of heterochromosomes in one form and not in others closely related? What has been the history of such differentiation where we have an unpaired heterochromosome or an unequal pair of heterochromosomes?

In *Metapodius* Wilson ('09) traces the origin of an unpaired heterochromosome and also of the supernumeraries to an irregular mitosis in which both idiochromosomes go to one daughter cell. In the *Diabroticas* the supernumeraries probably owe their origin to an irregular division of the unpaired heterochromosome. But in no case are we able to say when or how or why certain spermatogonial chromosomes became specially differentiated as heterochromosomes.

We have associated the *X* and *Y* chromosomes of the male with sex-determination, but possibly they have some other meaning and are only so correlated with the sex-determining mechanism that they have the same distribution as the sex characters. The case of the mosquitoes certainly indicates that we must study the heterochromosomes, apart from the idea of sex-determination, more intensively, in order to determine if possible why we have such chromosomes differentiated in some forms and not in others.

In such cases as *Anopheles* the sex characters may be supposed to be located in the unequal heterochromosomes or in the larger equal pair with which they are connected. This may also be true of the composite heterochromosomes of *Drosophila ampelophila* and *Hesperotettix* (McClung, '05). In the ordinary cases of an unpaired heterochromosome or an unequal pair, we may suppose the sex characters to be located in the heterochromosomes, but to be independent of the special differentiation, which may be entirely absent as in *Culex* and *Theobaldia*.

If we apply Morgan's new sex formula—

$$FmFm = ♀, \quad Fmfm = ♂,$$

to the cases under discussion, *m* may be located in any equal pair but *F* must be either in the heterochromosomes or in chromosomes correlated with them. Sex-limited inheritance in the male (Morgan, '10) would then seem to be possible only where unpaired or unequally paired heterochromosomes are present, and not in such cases as, *Anisoloba*, the Lepidoptera, *Culex* and *Theobaldia*.

It occurs to me as possible that heterochromosome differentiation may be directly related to sex-limited inheritance of certain characters. If so we should expect to find an unpaired or an unequally paired heterochromosome in the female of *Abraxas* where there is sex-limited inheritance in the female, and also to find sex-limited inheritance of some character or characters in the female of those echinoderms in which Baltzer ('09) and Heffner ('10) have shown that an unequal pair of chromosomes is present in the female. Apparently we have reached a point where the experimental breeder and the cytologist *must* work together in order to solve the problems of sex-determination, sex-limited inheritance and heterochromosome differentiation.

Bryn Mawr College,
December 20, 1910.

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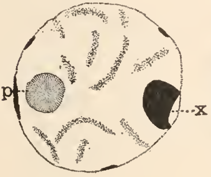
PRELIMINARY NOTE ON HETEROCHROMOSOMES IN THE GUINEA PIG.

N. M. STEVENS.

It is the purpose of this preliminary note on the spermatogenesis of the common guinea-pig merely to state some of the most obvious facts concerning the unequally paired heterochromosomes as they appear in the first spermatocytes. In the later growth stages a characteristic heterochromosome (Fig. 1, *x*)



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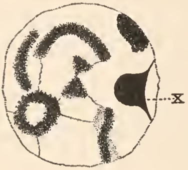

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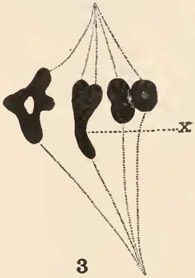

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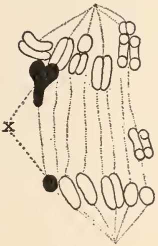

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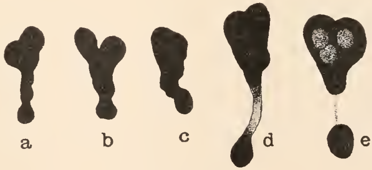

5

FIG. 1. First spermatocyte growth stage. X = the heterochromosome. Mag. 2,000.

FIG. 2. Prophase.

FIG. 3. First spermatocyte metaphase, tangential section of spindle showing the heterochromosome X.

FIG. 4. Anaphase.

FIG. 5. The heterochromosome; *a*, *b*, *c*, from sections of Flemming material; *c*, *d*, from acetocarmine preparations. Mag. 2,000 for all of the figures.

is easily distinguished both in acetocarmine preparations and in sections of Flemming material stained with thionin. The plasmosome (*p*) is even paler than the spireme. In Fig. 2, a prophase, the heterochromosome (*x*) is also shown. Fig. 3, a tangential section of a first spermatocyte spindle, shows the usual form of the heterochromosome in metaphase, and in Fig. 4 we have it divided into its unequal components. Fig. 5, *a*, *b*, *c*, shows slight variations in the metaphase form of the heterochromosome as seen in sections, and *d*, *e* the metakinesis of this chromosome, the large size of these two figures being due to the fact that the figures were drawn from acetocarmine preparations. Further details will be given later.

Bryn Mawr College,
January 5, 1911.

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BIOLOGICAL BULLETIN

THE METHOD OF CELL DIVISION IN THE DEVELOPMENT OF THE FEMALE SEX ORGANS OF MONIEZIA.[1]

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TABLE OF CONTENTS.

Introduction 123
Review of the Literature 126
Material and Methods 130
    Fixatives 132
Observations 134
    The Primary Anlage 135
    The Oögonial Period 141
    The Growth Period and the Behavior of the Nucleolus 142
    Synapsis 144
    Yolk Production 144
    The Ovarian Egg 145
    The Vitellarium 145
    The Female Genital Ducts 147
    Fertilization and Maturation 150
    Cleavage 152
Summary of the Evidence 155
Discussion 158
Comparison with *Tænia* 160
Conclusions 161
Literature References 162
Explanation of Plates 164

INTRODUCTION.

Cestode histology has been the subject of several investigations in recent years, some of the results of which are of far-reaching importance. Throughout the development of *Moniezia* Child has reported the regular occurrence of amitosis; he has described it as present not only in the somatic tissues but also in the germ cells. It was this latter phase of his results which occasioned the present work.

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[1] Zoölogical Laboratory, Princeton University, January 26, 1911.

There are inherent in the very nature of the problem involved in the recognition of cases of amitotic cell division as such certain difficulties which render the study of the question upon fixed material more or less unsatisfactory. Mitoses are, of course, well adapted for such a study, although they may be obscured in various ways. The presence of definite chromatin bodies and the absence of a nuclear membrane at most stages makes their recognition in properly treated material comparatively easy. But positive evidence of amitosis in fixed material is difficult to obtain. One cannot always be sure that a nuclear constriction is not a product of mechanical compression, *e. g.*, by yolk granules, or an artifact due to reagents rather than a stage in division. A strand of linin stretched across a nucleus with chromatin granules upon it often gives the appearance of a membrane dividing the nucleus amitotically (endogenous division?). Nuclear lobations are frequently seen in cells which are known to multiply by mitosis. Finally, a reduplication of nuclei for any of several reasons—disintegration, accessory sperm nuclei in egg cells, the male and female pronuclei themselves, lagging of cytoplasmic division behind nuclear mitosis, incompletely fused chromosomal vesicles, etc., easily presents a simulacrum of amitosis which is quite misleading. These and other factors[1] all render the amitosis problem difficult of solution.

Clearly, however, a definition of what one means by amitosis and of what he will accept as evidence of that process is imperative. Dahlgren and Kepner (p. 38) define amitosis as division "by a series of autoconstrictions of first the nucleolus, then the nucleus, and lastly the cytoplasmic body." The nucleus itself divides in three ways: the constriction of its body, the formation of a "nuclear plate" in the plane of division and subsequent separation, and the endogenous method of Child. Wilson (p. 114) describes the process as follows: "First the nucleus remains in the resting stage (reticulum), and there is no formation of a spireme or of chromosomes. Second, division occurs without the formation of an amphiaster. . . . The nuclear substance undergoes a division of its total mass but not of the individual elements or chromatin granules." It will be seen that these definitions are not sufficiently diagnostic to be of use in the work here undertaken.

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[1] Mitosis may be periodic and of very short duration.

For amitosis there is but one absolutely certain criterion, the observation of living material and subsequent study of material fixed under observation, and this is, of course, impossible in most cases. In the absence of such a criterion only complete series of all stages in the constriction and subsequent division of the nucleus and attendant cytoplasm would seem sufficient warrant for the assertion that amitosis is the prevailing form of division.[1] Even complete series are no assurance that part of the cells do not divide mitotically or that mitosis does not occur periodically. But given a complete series—assumed or proven—and taking no account of periodicity, etc., the interpetation of doubtful cases on the basis of the complete series follows logically. As will appear later such a complete series is extremely difficult to establish in the cestodes; its assumption, therefore, in the face of the above mentioned difficulties is ultra radical. The burden of proof is upon the shoulders of him who makes the assumption.

It is true, however, that a spirit of open-mindedness demands an impartial consideration of uncertain cases, incomplete stages and doubtful processes in the hope that an unprejudiced decision may be reached. The refusal to consider such cases is as unscientific as is the drawing of sweeping generalizations from them. In such a spirit of investigation I propose to set forth the facts as I see them in the case of the anlage of the female sex organs and its derivatives in *Moniezia*. I then wish to sum up the evidence for and against the occurrence of amitosis in this form and to consider various *a priori* suggestions which have been made by investigators to cover this case. Finally, I wish to make a comparison of these results with those of *Tænia* upon which I previously worked. In the development of the anlage of the female sex organs the history of the sex cells themselves, of the vitellarium and of the ducts and canals will be considered.

Interesting in this connection are the recent comments upon Child's position made by Boveri.[2]

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[1] To forestall criticism of this point, let me say that this is in no sense a preconceived hypothesis. I had worked on the amitosis question nearly two years before the significance of this contention was fully borne in on me. It is given thus early in the discussion merely to make the account more intelligible.

[2] "Zellenstudien," 6, p. 235.

"If, his (Child's) observations are to prove his contentions then it must be shown: (1) that the binucleate condition which he finds really depends on a division; (2) that about each of these nuclei a part of the protoplasm is marked off, and (3) that the cells so arising again divide by mitosis and possess the normal number of chromosomes."

Review of the Literature.

The literature of late histological researches upon the trematodes and cestodes deals with the general development of the flat-worm tissues, especially with the cuticle and the parenchyma. The more recent papers also touch upon the question of the occurrence of amitosis in the life history of the various cells.

Cestode histology according to this late work presents some very unique features. All investigators who have given the subject their attention seem to concur in the view that tissue growth here differs in character from the usual method of cell multiplication. All agree that karyokinetic figures are not to be found as numerously as in other tissues; some assert their entire absence; while one goes so far as to claim that nuclei arise *de novo*. On the other hand, some papers make no mention of amitosis, possibly due to the fact that the authors have not interested themselves with the problem. A comparison of the cases in which amitosis occurs is difficult to make because many reports, of thirty or more years ago, previous to the introduction of refined methods of technique, have not been substantiated by later work; but, nevertheless, it is safe to say that in no other group of animals have such unique growth phenomena been found by reputable workers. Granting for the moment that these phenomena have been correctly reported, one is compelled to ask what the relation is between them and the parasitic mode of existence which is universal among cestodes. While as yet there is no basis for speculation even on this relation, the modifications of gross structure due to parasitism are so profound in the cestodes that one may well be deterred from generalizing from observations of peculiar histological conditions in them.

Bugge ('02) was the first to note the absence of mitosis in cestode tissues in his work upon the flame cells.

Child in 1904 called attention to the lack of mitoses in the reproductive organs of the cestode *Moniezia* and asserted that amitosis takes its place. He further elaborated this conception and furnished more data in 1906 and 1907. As the object of this research is either to corroborate or to disprove his conclusions, and as it deals with them at length later, no further attention will be given to his earlier papers in this place. In a later paper ('10) he brings forward no new evidence but merely reiterates his former position. This work consists largely of an attack on my observations on *Tænia* ('09). He criticises my paper as presenting evidence of a negative character only; as being upon an entirely different genus and perhaps on different stages from those in which he observed amitosis; as prematurely generalizing from incomplete data; as using preconceived hypotheses; and finally, as being the work of a novice who "is likely to be guided to a greater or less extent by the views of his instructor." I may venture a reply to the main points of this list. I regret to have my paper taken solely as a criticism of Child's work; it recounted the facts as I believe them to exist in *Tænia;* its field, it is true, is limited to the oögenesis and cell multiplication of certain somatic tissues in this worm, but no attempt is made to apply its conclusions to any of Child's work not upon a similar field (a fact which he does not seem to have appreciated). I found no evidence of amitosis in that field and as Child had covered a similar one it was not only natural but necessary that I should compare my results with his. As to the negative character of my evidence, I would ask whether the failure to find evidence of a certain process, using proper methods and exercising due diligence, is not positive evidence of the lack of that process. Of what more would positive evidence consist? As to my not having studied the proper stages little need be said; no method of procedure other than beginning at the scolex and working backward ever occurred to me. As to the fact that I studied a different genus, he says, "Such records (referring to the results published in his previous papers) cannot be controverted . . . by observation upon another genus and species. . . ." His views upon this point, it seems, have undergone a change. In his "General Conclusions" to "Amitosis in Moniezia" ('04) he said, "It is scarcely possible that *Moniezia* differs from the majority of other forms in the amitotic multiplication of nuclei." There are, however, a considerable number of differences in the results obtained in the two allied forms, differences which I hope to make clear in the course of this discussion.

Spengel in 1905 before the Deutsche Zoologische Gesellschaft[1] stated that neither mitotic nor amitotic cell division had been observed in cestode development.

C. v. Janicki ('07) in an important paper upon the embryonic development of *Tænia serrata* states that he finds no pronounced division figures up to the growth period of the oögonia, but during the growth period he describes spiremes; he does not suggest amitosis as the explanation of his failure to find mitoses but merely says that the divisions must have passed very quickly. In the cleavage stages achromatic parts of the division figures are often hard to demonstrate.

In so far as it touches the same points my own work ('09) on this form agrees with that of v. Janicki. I recorded my failure to find amitosis and noted that mitoses are more infrequent than one would expect.

In 1908 appeared the paper by Young upon the histogenesis of *Cysticercus pisiformis*, the cysticerus stage of *Tænia serrata*. This paper is revolutionary indeed, and agrees in practically no cytological points with the work of v. Janicki and myself. Not only does he accept Child's conclusions and their bearing upon hereditary problems but he refuses to subscribe to Virchow's dictum "omnis cellula e cellula." He believes that nuclei arise *de novo* in accordance with a tentative hypothesis, of which his statement is as follows: "I believe that the nucleus in these forms is not a morphological but a physiological entity; that the nuclear granules are fundamentally the same as the remaining protoplasm of the cell, but are differentiated therefrom under physiological conditions which we do not at present understand; that these granules are perhaps reserve material stored up in the nucleus for future use, the entire cell body being occasionally converted into a nucleus; and that the nucleus varies in structure from time to time in response to the varying physiological demands made upon it. . . . Further, if my interpretation of my observations be correct, then distinction between germ and somatic plasm is obviously impossible; a special vehicle for the transference of heredity qualities is entirely wanting; such qualities must be transmitted by the undifferentiated protoplasm; cell lineage is manifestly lacking; a mosaic theory is plainly untenable; and the fate of any given embryonic element—whether it shall form parenchyme, muscle, nerve, etc.—must be determined by physiological causes alone." In an appendix referring to Child's later papers Young says that he has verified Child's conclusions upon the reproductive organs of *Tænia serrata*. This, of course, is exactly opposed to the work of v. Janicki and myself upon *T. serrata*. I am not aware that he has published any further account of these more recent observations.[1]

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[1] See Child, '07*a*, p. 277.

Balss ('08), in his extensive researches on the development of the sex ducts of *Anoplocephala* and *Solenophorus* did not, I believe, direct his attention to the method of nuclear division, nor do his figures throw any light upon the question. His paper is extremely valuable in making a study of the early stages of sex organ formation.

The last of the important papers on cestode histology is that of Spätlich ('09) on *Tetrabothrius*. This author states that he finds cases of amitosis and gives several figures of the cells in question. He does not doubt that the cells are in the process o amitotic division and certainly that is the easiest explanation of the figures; but to one skeptically minded the proof will not appear as conclusive. The cell in his Fig. 54, for example, might easily be interpreted as a case of compression; while there is no evidence that either of Figs. 54, 55 and 56 (drawn out nuclei, becoming dumb-bell shaped) would actually have completed the division. Nothing is said of a complete series of stages. Furthermore, these are cases in the development of the "Dotterzelle" and may perhaps have been at the end of their life cycle.

In glancing over the list of tape-worms in which amitosis or other irregularities of cell multiplication have been described, one is struck with the fact that according to the classification usually given only members of one family, the Tænidæ, are included in it. This, of course, may be the merest coincidence owing, perhaps, to the fact that members of the Tænidæ are very readily secured. The warning uttered by Young with regard to applying conclusions drawn from cestode observations to other groups, is, however, more strongly emphasized. Not only are the results which he had in mind deduced from a single class of animals which are degenerate in a high degree, but they are drawn from a single family of that degenerate class.

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[1] In *Science* of February 17, Young gives a very brief résumé of this work. Although he finds no mitosis, too few amitoses occur to account for the necessary cell multiplication; he thinks that the "increase is partly due to development of nuclei either de novo or from chromidial extrusions of preexistent nuclei."

Material and Methods.

At the suggestion of Professor E. G. Conklin, the genus *Tænia*, upon which I had begun investigation with a view to obtaining further evidence as to the occurrence of amitosis in cestode tissues, was dropped for the present as a subject for research in favor of *Moniezia*. The results which have been obtained upon this latter genus by Child are of such unusual nature compared with those of other forms that a reëxamination would seem desirable. In the light of my investigations upon *Moniezia* and of Child's recent criticism I have again gone over my old material of *Tænia* as well as some newly prepared.

In making these studies I am indebted to Professor E. G. Conklin and to Professor Ulric Dahlgren for much kindly assistance. My thanks are due to the management of the Wistar Institute of Anatomy for assistance in obtaining material. Not only were the facilities of the laboratory extended me for making collections but my first lot of material was obtained and fixed by Dr. Helen Dean King. To Dr. King I also owe thanks for a number of technical suggestions. Professor C. M. Child, of the University of Chicago, has also kindly placed facilities at my disposal for obtaining specimens.

Specimens of *Moniezia expansa* and of *M. planissima* have been secured and fixed in a variety of fixing fluids: Flemming, Hermann, Graf's chrom-oxalic, saturated aqueous solution of corrosive sublimate, formol-sublimate,[1] Kleinberg's picro-sulphuric, Zenker's fluid, Zenker plus an excess of acetic acid, and a modification of Zenker's fluid by Dr. King.[1]

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[1] Physiological salt solution saturated with corrosive sublimate to which a few drops of neutral formol are added.

None of my sections have been cut over 4 micra thick; many of them are thinner. Pieces of the worms about 3–6 mm. in length were embedded serially beginning at the scolex or the neck region and extending back some 35–40 cm. usually, including the beginning of the proglottids with fully formed embryos. Pieces were then selected at intervals and cut; later, if examination warranted, the intervening pieces were cut. In this way practically one entire worm has been cut and studied, while from a considerable number of other worms incomplete series have been made as described. I have used both longitudinal and cross sections.

A number of staining methods have been tried on *Moniezia*, some of which gave good results, while others succeeded only indifferently. I have been unable to get good preparations with Kernschwarz, although this stain served me well on *Tænia*. The other stains used include Delafield's hæmatoxylin, hæmalum, thionin, Bismarck brown, iron hæmatoxylin alone and counterstained with Lichtgrün or Bordeaux R, a modification of Conklin's picro-hæmatoxylin method for staining eggs *in toto*,[2] Ehrlich-Biondi, and the Flemming triple stain, safranin gentian-violet and orange G.

Of these stains, iron hæmatoxylin is the best general stain and most reliable, but for certain phases of the work it is not sufficiently differential. I have found picro-hæmatoxylin and the safranin gentian-violet mixture especially useful in connection with it. (In most cases I find that the orange G of the triple stain detracts from rather than adds to its value.)

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[1] Dr. King uses two solutions, as follows: (1) Four per cent. potassium bichromate; (2) corrosive sublimate, 4 gr., glacial acetic acid, 20 c.c., water, 76 c.c. The two solutions are mixed equally and allowed to act for one to two hours as the mixture penetrates rapidly. For convenience, I shall hereafter call this mixture "King's fluid."

[2] This is an application of the *in toto* method to sections containing eggs. It consists of mordanting the sections for a short time—too long is injurious—in aqueous picric acid solution. After staining the sections may be decolorized in this same solution and then blued in tap water. It gives the desired differentiation between yolk and chromatin, although not with the characteristic colors of Conklin's stain.

Fixatives.—To show exactly the various effects of the fixatives used and to show that the results obtained are not due to the exclusive use of one reagent, I wish to give here a detailed statement of those effects.

In his work on *Moniezia* Child used some eleven fixatives of which the most successful were Hermann's fluid, saturated aqueous solution of corrosive sublimate, and Graf's chrom-oxalic mixture. I, therefore, used these three fluids and in addition certain others which my own experience suggested. These were, as previously stated, Flemming's and Zenker's fluids, Zenker plus an excess of acetic acid, and formol-sublimate. Dr. King fixed some of my first lot of material in her modification of Zenker's fluid. I have no hesitation in saying that this fluid gives the best results for *Moniezia* of any which I have tried. Subsequently, I myself have used this solution with equally satisfactory results.

The results of my experiments with the various fluids follow. Chrom-oxalic is not, I find, a good general fixative for *Moniezia*. The general cytoplasmic structures, parenchyma, etc., are not well preserved and the achromatic substances of the nuclei are dissolved out. The chalk bodies[1] also are not retained. Testis cells are more satisfactorily fixed, as are also the cleavage cells and young embryos. But in the case of spermatozoa and the young female germ cells the mixture is not successful. Nuclei are everywhere swollen, but in these special cases they are also distorted. I should not care to trust any results based on the observation of early oögonia in my chrom-oxalic material. Many of Child's results are founded on chrom-oxalic material; doubtless he had greater success with this mixture than I did.

Hermann's is a good fixative for the developing germ cells, both male and female; for parenchyma the results are slightly less satisfactory. Nuclear contents are well preserved in this solution—much better than in the preceding one. It also gives a good preparation of the cuticle. With Flemming's, Hermann's shares the drawback of fixing the calcareous bodies so that they stain black with certain dyes. This is a serious drawback to the use of osmic acid fixatives, for the chalk bodies are so numerous as to obscure much of the development of the reproductive organs.

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[1] As part of their "generic diagnosis" Stiles and Hassal give this statement, "Calcareous bodies absent from the parenchyma." They, however, studied only sublimate material.

The remarks concerning Hermann's apply with equal force to Flemming's although the latter gives a somewhat more nearly perfect fixation considering the tissue as a whole. For the cuticle Flemming's gives better results than any other reagent I have tried.

My experiments with corrosive sublimate in aqueous solution have proven a complete failure. Circumstances connected with the experiments were such, however, that I am not disposed to regard my results as typical; had I not already had a great number of successful preparations I should have given it further trial.

With formol-sublimate I have obtained good preparations. It gives quite good nuclear and cytoplasmic fixation as well as a very fair preservation of the parenchyma. Nuclei and cytoplasm stain dark after it; chromatin is well fixed but achromatic structures are not easily differentiated owing to the homogeneous condition of the nuclei; cytoplasm, too, is of a dense homogeneous nature not unlike that of certain nerve cells. Calcareous bodies are not present in sublimate material. Formol-sublimate is, however, a good general fixative for *Moniezia*.

Picro-sulphuric, while giving somewhat better results than aqueous sublimate (the young embryos are fairly well preserved) is also a failure. It gives a better fixation of the parenchyma than of other tissues.

There yet remain the Zenker solutions. These give the best fixations, and of them the modification by Dr. King is the most satisfactory of all the fluids I have used on *Moniezia*. I find, too, that I am able to get better staining reactions on the material fixed in this solution. My study has been made chiefly on this material but I have in every case compared the results with those of the other fixatives. The fixations by chrom-oxalic, Hermann's, formol-sublimate, and King's solution all depend on different actions; therefore, it would seem that the phenomena common to these four reagents cannot be viewed as artifacts due to fixation.

Observations.

Morphological discussions are without the scope of this research, but a knowledge of the derivation is helpful to an understanding of the development of the structures dealt with. Excluding the cuticle—the writer has not given much attention to the "ectoderm question," but his view, in so far as his observations warrant one, is that the cuticle also is of parenchymal origin—all of the adult structures of the tape-worm are derived from the parenchyma. Parenchyma is physiologically similar to embryonic mesenchyme in that from it are derived muscle fibers, flame cells, etc. The testes arise from the parenchyma first in the region of the longitudinal excretory tubes and later appear medianward, occupying the side of the worm commonly designated as dorsal. The animal is incompletely protandrous for the ovaries arise later and many of the testes have completed their development and degenerated before the female germ cells have progressed very far. The last of them do not complete their cycle until most of the ova have already been fertilized but all ultimately degenerate and their place is taken by the branches of the uterus.

The first anlage of the female reproductive apparatus arises also in the neighborhood of the excretory tubes and grows both laterally and medianward. Its lateral growth gives rise to the seminal receptacle and the vagina. Its median growth is the beginning of the oviduct, which later branches to form the uterus, of the vitellarium and shell glands, and of the ovary. Thus the entire female apparatus arises from an originally single, indifferent anlage. This shortly differentiates into the separate anlagen each of which from now on pursues its own individual course. That of the ovary is the innermost part of the original anlage. It develops its investing membrane and begins a growth, due to the growth of the germ cells composing it, which ends only when the ova have all attained their complete size and developed the needed amount of yolk. These in a rapid passage through the oviduct are fertilized and enclosed in a thin shell. Maturation is completed in the uterus where cleavage occurs and early embryonic life is passed. When all of the ova have been fertilized the reproductive mechanism except the uterus degenerates. The entire process of development from primitive germ cells to fully formed embryos is one of comparative simplicity and is quite easily followed with the exception of the cleavage stages. These are difficult to understand in section, and whole mounts of them are not readily prepared.[1]

The vitellarium arises from the middle part of the primary indifferent an age, and in its early stages cannot be distinguished from the ovary other than by its position. But its growth is of a different character and soon permits of a distinction between the cells of the two regions. It acquires the character of a gland and thereafter its activities are those connected with secretion.

The development of the various female organs will now be considered in detail.

The Primary Anlage.—The indifferent or primary anlage begins merely as a thickening of cells median and slightly ventral to the longitudinal nephridial canals. This thickening is not marked off in any way from the surrounding parenchyma; in fact there is a gradation from one into the other. There is a slight difference in the character of the cells of the anlage and of the adjacent parenchyma but it is only slight. The cytoplasm is perhaps a little more dense in the anlage, and the nuclei show evidence of greater activity. The cells of the parenchyma are clear and usually have but one nucleus. The cells of the anlage stain darker, and their nuclei frequently possess two or more nucleolus-like bodies, of which probably only one is a true nucleolus, and show a more or less distinct reticulum, except when fixed with chrom-acetic. The ratio of nucleus to cytoplasm is at first somewhat greater but soon becomes less in the case of the anlage than of the parenchyma. Nuclei in the former are often somewhat smaller than in the latter.

To avoid confusion with regard to my use of the term "cell," I will here state my view of the cellular nature of the parenchyma. I do not consider the parenchyma to be a syncytium as that term is generally interpreted. Nuclei are always surrounded by a definite amount of cytoplasm, but that cytoplasm is lacking in a definite limiting membrane or cell wall.[2] The remainder of the parenchyma tissue is made up of loose intercellular material (of cellular origin, of course). Thus a cell consists of a nucleus and its attendant cytoplasm supported by a large mass of intercellular material. Usually the cells are somewhat distant from each other. Should two or more of them come to lie closely side by side, it is conceivable that their cytoplasmic masses might easily fuse and a true syncytium be formed. It will be seen that this is a possible explanation of binucleate parenchyma cells where such occur (Fig. 2). The cellular character of the primary anlage is similar to that of the parenchyma, although, of course, the anlage has very little intercellular material and many cells.

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[1] Cf. for this general development photomicrographs I. to IV.

[2] My former statement that "the parenchyma cells of *Tænia* have definite cell boundaries" was not intended to mean that they have cell walls; I have not observed that the two genera differ in this respect.

Child describes the testes as arising from visibly differentiated muscle cells; he did not, however, observe that muscle cells take part in the formation of the ovary, "probably because these cells do not occur in the region where the ovaries develop" (Child, '06). Muscle cells certainly do occur in the region of the ovaries[1] but I have never seen any evidence that they take any part whatever in the development of the female germ cells.

Since the primary anlage arises from a parenchyma which has no visible differentiation there would seem to be no visible basis for a continuity of germ plasm here. The expression "primordial germ cell" has little meaning therefore when applied to a cestode for there is no apparent difference, except of position,[2] in the cells which will go to form the ova and those which will form the vitellaria or the walls of the ducts.

The primary anlage grows both outward toward the edge of the proglottid and inward toward the ventral surface. Its outward growth is in the form of a cord of cells being extended to the exterior. Before the solid cord reaches the exterior its middle portion becomes hollowed out to form a lumen. This lumen subsequently develops a cuticle which to all appearances is just like that on the surface of the body. This outward growth becomes the vagina and the receptaculum seminis. Its development takes place somewhat more slowly than that of the inward proliferation of cells, due, doubtless, to the fact that its function is exercised only relatively late.

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[1] See photomicrographs for circular and longitudinal layers; scattered fibers also occur.

[2] This difference of position, however, is a very constant one. The cells from which the ova develop are early marked off by their position and never take part in any other development. The facts are not opposed to the continuity theory; they simply afford no evidence for it.

The inward "proliferating area gradually extends somewhat toward the median plane and somewhat toward one surface of the proglottid known as the ventral surface. . . . As the inner and ventral end of the proliferating area approaches the inner layer of circular muscles it spreads out into a flattened somewhat disc-like area exactly as if it had encountered resistance to its growth in the original direction and so had begun to spread out in other directions."[1] A little later the posterior portion of the anlage of the ovary becomes the anlage of the vitellarium which develops about the middle part of the oviduct. This origin shows that morphologically the vitellarium is part of the ovary and gives credence to the view expressed for similar cells of other animals (test cells of ascidians, etc.), that the cells are rudimentary eggs.

There are two regions concerning whose method of growth it is especially important to have definite knowledge. These are the primary anlage and the early embryos. Concerning the former there are four possibilities as to the growth of the cells: origin *de novo*, by migration from the surrounding parenchyma, and by cell division direct and indirect. The first of these as Child remarks ('10, p. 112) is "an alternative which most of us would hesitate to accept without proof of the most conclusive character."

Migration of nuclei to this region, if one could show that it occurred, would explain the relative lack of mitoses in the anlagen. It would then be necessary, of course, to seek for mitoses or amitoses in the neck region to determine the method of origin of the cells. But actual evidence of migration is as impossible to obtain as is evidence of amitosis. At best only indirect evidence of it can be secured.

If abundant divisions occurred there would be little probability of migration, although it might occur. Abundant divisions are not to be seen, however. On the other hand, the fact that many of the nuclei in the primary anlage are smaller than the parenchymal nuclei suggests that they have divided. Still a third line of evidence is obtained by a study of the number of nuclei in given regions before and after the formation of the anlage. To make such a study it is not sufficient to count the nuclei in a single section of each proglottid and compare the counts directly, for the proglottid grows in three dimensions. Either the entire number of nuclei in all the sections to be compared must be counted or the ratio determined by a mathematical calculation based upon counts from different fields of the two sections, measurements of the inner parenchymal ellipses (that part of the parenchyma enclosed by the circular muscle layers) and the number of sections of the proglottid.

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[1] Child, '07*b*, p. 99.

Such a calculation has been made and its results are given in the following table. While the problem does not lend itself fully to the rigor of a mathematical treatment, it is believed that the results are approximately accurate, at least sufficiently so for the sake of a comparison. In each case two proglottids from the same chain, one near the head, the other farther back, are compared; by nuclear ratio is meant the ratio of the number of nuclei within the circular muscle layer of the one to that of the other; the volumetric ratio represents the ratio of the volumes of the parenchyma within the muscle layers for the same proglottids.

Case.	Stage of Development.	Nuclear Ratio.	Volumetric Ratio.
A1 A2	Testes formed, ovary anlage separate. Growth period.	6 : 10	7 : 10
B1 B2	Ovary anlage just separated. All organs formed, merely growing.	1 : 2	1 : 5
C1 C2	Primary anlage stage. Late growth period.	1 : 7	1 : 17
D1 D2 D3	Early primary anlage. Ovary nearing growth period, testes almost all formed. All organs formed.	2 : 9 : 10	2 : 9 : 11½
E1 E2	Early primary anlage. Testes formed, ovary in growth period.	1 : 6	1 : 7
F1 F2	Primary anlage stage. Testes formed, ovary in early growth period.	1 : 7	1 : 7

Of the six cases here given, believed to be fairly representative, in only one, A1 and A2, have the nuclei increased in greater proportion than the volume, and even here the increase is only slightly greater; in two, D1 and D2, and F1 and F2, the two ratios have kept pace with each other; while in four, B1 and B2, C1 and C2, D1 and D3, and E1 and E2, the nuclear ratio has fallen more or less behind the volumetric. Now evidences of division are no more—and no less—common in the parenchyma cells than in the anlage so the result of these four cases favors somewhat the notion of migration of the parenchyma nuclei to the anlage for there are relatively fewer nuclei in the older proglottids and it is hardly probable that degeneration, the opposing factor, would play a part in the decrease in the number of nuclei in as young proglottids as these are. But on the whole the evidence for migration is not strong; certainly this factor cannot have been the important one in the formation of the anlage of the female organs.

It would seem, then, that here as is general throughout the animal kingdom germ cells must arise by cell division. It is the inconspicuousness of these processes that occasions the present discussion.

Mitosis unquestionably occurs. Child records and figures occasional cases. Although he holds that they are too infrequent to account for the cell proliferation he finds them in nearly every stage of development. He has, however, never found them in the early stage of the duct formation, in the primary anlage, unless his recent account of a single individual consisting of scolex, neck region, and a few of the youngest proglottids in which nearly every nucleus is dividing mitotically may include such cases. He has given no definite statement of the stages included in this worm, but certainly the primary anlage appears in the proglottids which are quite young. In these earliest stages I have found a considerable number of mitoses although they are by no means so abundant as to be seen upon a cursory examination. Let me call attention to the fact that in almost any tissue the ratio of resting to dividing cells will be found to be very great. This ratio is a surprising one.

In the oögenesis of *Planorbis*, a form in which divisions are of the usual mitotic type is shown in a research, as yet unpublished, by Conklin, the division figures are very difficult to find. One may examine section after section without seeing a single case of mitosis. Although I have not made a statistical study of the two cases I am of the opinion, from the examination of the slides which Professor Conklin has kindly shown me, that division figures are more rare in the oögonia of *Planorbis* than in those of *Moniezia*. The ratio of resting cells to dividing cells is large. Yet there is no evidence for the occurrence of amitosis here. Another better known example is found in the oögonia of *Ascaris*. Any one who has searched for oögonial divisions in this animal knows how few cells show them in proportion to the number present.

As development progresses mitoses occur as stated up to the growth period, but, it is true, not as frequently as one would expect from conditions in other tissues. I do not find that the figures are always as easily recognizable as are mitoses in many tissues.

That mitoses occur throughout the development of the female germs cells in considerable numbers, there is not the slightest doubt. The evidence for this is positive. Furthermore, they always occur in the peripheral, *i. e.*, the growing portion of the anlage, a very significant fact. It requires merely a little careful search upon properly fixed and stained material to discover them. Tissues fixed in King's fluid and stained with the Flemming triple stain are especially favorable for finding the mitotic figures. They do not occur as frequently as one would *a priori* expect, but, in the light of the fact which will appear immediately, *i. e.*, that there is no good evidence for the occurrence of amitosis, we are merely forced to readjust our ideas of the number of divisions necessary to produce a tissue. The mitotic frequency has not been sufficiently considered. I regard it as also highly probable that periodicity of division is an important factor in tissue production.

There are to be seen here many cases of nuclei which lie close beside one another with only a narrow layer of cytoplasm between them. There have been some few cases in which no layer could be distinguished, but the character of the material is such that this easily lies within the limits of observational error. These cases may be considered as evidences of recent division; they are not, however, distinctive of either direct or indirect division. If there were abundant evidence of the former they would be regarded as adding to it. On the other hand, if spindles were very numerous this arrangement in pairs would be looked upon as the final stage in the division of the cells by mitosis. Cases of juxtaposition of nuclei are seen in Figs. 2, 12 and 17.

One may occasionally see in some sections a few nuclei darker than their neighbors (Fig. 18) scattered among the pre-oögonia. They have no visible significance; they differ from other nuclei only in being darker. I have never seen one half of a nucleus darker than the other. I may here add that there is never a dark body, a "Nebendotter" occupying a portion of an ovum as in *Tænia*.

Rarely, indeed, does one find constricted nuclei in my preparations. Shrunken and irregular nuclei—artifacts—occur; strands of linin across a nucleus often resemble the formation of a plate (as in Figs. 41 and 42); the edges of adjacent nuclei overlap and sometimes the overlapping parts are so thin that the true condition is not at first apparent; but the various stages of plate formation, constriction, etc., and especially of "endogenous" division as set forth by Child I have never seen. One does, however, occasionally find a dumb-bell-shaped nucleus; but so infrequent are they that I am unable to determine any significance for them.

I find myself, therefore, unable to confirm Child's conclusions for the various reasons given. His position, however, that the "relative frequency of mitosis and amitosis in certain species, and even in single individuals may vary greatly according to conditions" ('10, p. 116) is an unassailable one with our present data.

The Oögonial Period.—From the disc-like anlage on the ventral side of the proglottid upward projections or follicles develop and give to the ovary its characteristic form.[1] About the time of full development an enveloping membrane appears probably as a differentiation of the surrounding parenchyma. For the female germ cells from the differentiation of the ovarian anlage to the formation of the membrane of the ovary the term "pre-oögonia" has been (aptly, I think) suggested by Professor Dahlgren (see Figs. 9–14). It is only at the end of the pre-oögonial period that the cells which are to form ova are certainly distinguishable from all others. The convenience of the use of this term will be apparent. The formation of the follicular membrane seems to mark the time of cessation of active proliferation on the part of the oögonia. I have seen no sign whatever of cell division in stages following this. There are no longer many parenchymal elements to be seen in the ovary and each oögonium becomes sharply marked off from its neighbors. The subsequent growth of the ovary is merely that attendant upon the growth period of the oögonia. The cell divisions are, of course, limited to the maturation and cleavage divisions.

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[1] Cf. Photomicrograph III.

The Growth Period and Behavior of the Nucleolus.—The growth period in the cestode as in most animals is relatively long; many more proglottids have the ova in this condition than in any other previous to the cleavage stages. The actual duration of the growth period is probably three or four months.[1] It is well known that sheep older than yearlings are rarely infested with tape-worms. The parasites occur only in the young, but nevertheless they attain a length of 4–5 meters in the case of *M. expansa* and of 2 meters in the case of *M. planissima*. Developing embryos first occur at a distance of 100–110 cm. from the head; the primary anlage appears 7–8 cm. from the head; and the growth period of the oögonia begins about 20 cm. from the head. This short duration of the stage in which multiplication of the oögonia is taking place must be kept in mind when investigating the method of cell division here. To Child's description of the growth period little can be added. Briefly, there is accomplished during the period the great growth of the eggs, synapsis, the formation of the yolk globules, and certain contemporary changes in the nucleolus to be described immediately. During this period of no divisions the oögonia after synapsis are characterized by a minimum amount of chromatin, faintly staining, distributed peripherally, although strands may reach the nucleolus.

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[1] These time limits are tentative for I have been able to approximate them, only

Concerning the structure of the nucleolus more can be seen during the growth period than at any other stage of development. In favorable preparations, the nucleolus in oögonia, oöcytes and segmentation cells shows a peripheral portion staining violet with the triple stain, and a central portion, the so-called vacuole, staining with safranin. During the growth period additional details appear. These are (Figs. 28–30), within the vacuole, a small more or less refractive body, the endonucleolus (cf. Montgomery, '99), staining very dark with gentian violet, a reticulum supporting this body, and in the outer peripheral portion of the nucleolus certain darker bodies. Certain appearances here very much resemble the conditions described in the karyosome cycle of *Diplodiscus* by Cary ('09). There is, however, no parallel in the development.

Only one true nucleolus is present in a cell. Besides this there are frequently one or more chromatin bodies, karyosomes, which with iron hæmatoxylin stain like nucleoli. But the gentian violet or Ehrlich-Biondi preparations never show more than a single true nucleolus.

As to the nature of the true nucleolus there is not much evidence. Young deplores the use of the term "nucleolus" and calls the structure a "nuclear granule." It is from the nuclear granules, he holds, that the nuclei develop; the granules in turn arising *de novo* (probably) from a common "cytoblastema." "Containing, as they do in many cases, most of the staining matter of the nucleus, they represent rather the chromatin than the true nucleoli." I am obliged to dissent from this view; they are without doubt true nucleoli, as differential staining shows. Nevertheless, in addition to their nucleolar character they may well serve as chromatin reservoirs during the resting period of the nucleus. Indeed, the peripheral portion stains with gentian violet just as does chromatin, and the darker bodies easily suggest chromatin masses. The fact that the nucleoli increase in size like the other elements of the cell during synapsis is explained by the swelling of the vacuole, for this occurs markedly; in many cases several vacuoles are present. Furthermore, the nucleoli are always in intimate connection with the spireme, and of course they disappear when mitosis comes on. C. v. Janicki ('03) finds that in the trematode, *Gyrodactylus*, the chromatin goes into the nucleolus during the resting stage.

Synapsis.—Whether or not chromatin actually comes from the nucleolus, the nucleus in the early part of the growth period acquires an abundance of chromatin and a spireme stage at once ensues. The spireme, in contact with the nucleolus, develops rapidly and becomes massed on one side of the nucleus just as in the "bouquet" or "synapsis" stage of the first maturation prophase. Although some time intervenes between this and the prophase of the first oöcytic division, I regard this as probably a true synapsis. What seems to be conjugation of the chromosomal loops occurs (Fig. 26) and all the characteristics of a synapsis are present. Furthermore, precocious synapses are not unknown (*e. g.*, see Montgomery on *Euchistus*, '01). Beginning in the medullary portion of the ovary, synapsis spreads rapidly over the entire organ, involving nearly all the oögonia at once, although they are in different stages in different parts. At the same time the cytoplasm is increasing in amount but there is no evidence of a causal relation between the two phenomena except that they are synchronous. The nucleus is as a rule excentrically placed and the "bouquet" is frequently found on the side of the nucleus next to the greatest cytoplasmic mass, but there are many exceptions to this arrangement. Following the "bouquet" stage the spireme spreads throughout the nucleus, loses its property of staining very densely and takes on the appearance of the typical resting nucleus about to undergo maturation. It remains in this stage for a relatively long period during which yolk production is accomplished and the ovum attains its full size.

Yolk Production.—Yolk production rarely begins before the "bouquet" stage has passed off. Usually upon the same side of the nucleus as that where the "bouquet" occurred, but always in a part where the cytoplasm is abundant, an area staining more deeply than the rest of the cytoplasm is visible. "It is comparable to certain of the differentiations which have been called yolk nuclei in other eggs and its appearance is followed almost immediately by the formation of yolk granules which are contained in the egg cell itself" (Child). Often two or more of these yolk-producing areas are to be seen in the same ovum.

In certain favorable material I can see a central dark granule about which appears a clear brownish court (iron hæmatoxylin material) and outside of this the yolk granules develop (Figs. 31 and 32). Whether this is general I am unable to decide; probably, it is. Child thinks, and it seems a logical conclusion, that the bouquet stage is responsible in some way for the yolk production. Similar conditions have been described by Janicki for *Tænia serrata*, and by several authors, particularly Goldschmidt, for trematodes. Goldschmidt[1] thinks that during the spireme stage a separation of somatic and germinal substance occurs and that the "trophochromatin" escapes from the nucleus to reappear later in the cytoplasm as yolk nuclei. Somewhat analogous accounts have been given for the eggs of animals of other groups.[2]

The yolk of *Moniezia* differs from that of *Tænia* in that in the former it occurs as spherules filling the entire cytoplasm while in the latter there is only a single mass (in the earlier stages more) as large or larger than the nucleus lying in the cytoplasm.

The Ovarian Egg.—The end result of these processes is the ovarian egg. It is irregular in shape due to crowding by surrounding cells and neighboring eggs, and contains a large germinal vesicle located asymmetrically. Filling the cytoplasm more or less completely are yolk globules of various sizes. The nucleolus is now comparatively small and is located near the periphery of the nucleus. Chromatin is distributed throughout the nucleus in the form of fine granules attached to a linin reticulum. I cannot say that centrosomes and centrospheres occur but I have seen some structures resembling them (Fig. 34). In this condition the egg is ready for passage through the oviduct where fertilization occurs. The lumen of the oviduct where it enters the ovary is much smaller than the diameter of the egg so that pressure is exerted on the egg during its passage causing it to change its shape. After reaching the uterus it regains its spherical form.

The Vitellarium.—Early in the development of the anlage of the female sex organs there is set aside a certain portion of the cells to form the vitellarium and shell gland. It lies posterior to the part which becomes the ovary arising from a group of cells branched off from the middle portion of the anlage—that portion which becomes the oviduct. The medullary portion develops first becoming the shell gland; from the periphery of this newly differentiated anlage are proliferated cells which become the vitellarium proper.

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[1] See Janicki, p. 695.

[2] Cf. Conklin on *Crepidula*, '02.

The morphological relations of this organ and the ovary suggest that the vitellaria are fundamentally ova specialized along another line than reproduction. This view, I think, is clearly supported by the evidence.[1] I have, therefore, endeavored to find a parallel in their development; it is not, however, a very close one. They agree in that they early pass through their division stages and do not proliferate during the production of yolk. (I have seen only a single case of division, and that not clear, in a nucleus of that period.) They differ in that the oögonial nuclei continue to increase in size long after the vitellarium nuclei have reached their full growth and the former are always more chromatic than the latter. The vitellaria never go through the synapsis stage and the method of yolk formation is unlike that of the oögonia. Small spherules which fuse with one another are formed in the cytoplasm. The mass thus arising grows larger pushing the nucleus to one side. The completed cell looks not unlike a fat cell of the "signet ring" type.

Like the ovary the vitellarium at the time when cell multiplication stops develops a membrane separating it from the other organs of the complex. Previous to this stage the cytoplasmic boundaries have not been as clearly defined as those of the other cells of the primary anlage, but now they become quite distinct and the cells more dense. The nuclei develop more chromatin, not in the form of a spireme as in the case of the ova, but as enlarged granules giving to the nucleus the appearance of several nucleoli. Sometimes strands may be seen extending from the nucleoli out to the periphery where the yolk masses are forming.

The further history of the cells of the vitellarium is of great interest from the standpoint of the histology of secretion but is outside the scope of the present problem.

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[1] Cf. Child, '07*b*, p. 113.

Although the vitellarium and the ovary arise from the same anlage and at first are distinguishable only by their position they soon acquire histological differences. The nuclei of the former contain very lightly staining chromatin in the form of a reticulum but there is a large nucleolus. It frequently occurs that there are two or more nucleolar-like structures, karyosomes, in a nucleus. The appearance of the nucleus itself is that of an almost empty court surrounding the nucleolus or nucleoli. The nuclei of the oögonia, on the other hand, do contain a reticulum, rather lightly staining, it is true, but consistently present. While the cytoplasm is more indefinite in amount in the vitellarium it can be seen that the ratio of nucleus to cytoplasm, if the inclusions are not taken into consideration, remains much more constant; if the yolk mass be considered the second term of the ratio is much increased. At no time after they become clearly distinguishable are the cells as large as the oögonia, and at maturation the oöcytes are more than twice their size.

As to cell multiplication I am convinced that after a comparatively early period division ceases. During this early period clear cases of mitosis are to be seen, but, it is true, as Child says, less frequently than in the ovary. On the other hand, the arrangement of nuclei in pairs is, perhaps, more in evidence here and indented nuclei are somewhat more numerous. The indentations do not, however, give a series of autoconstrictions from slight indentation up to complete division. If mitosis is not clearly proved as the sufficient cause of cell multiplication amitosis is certainly less so; for there is positive evidence that some mitoses do occur, but for amitosis there is only negative evidence.

The Female Genital Ducts.—From the same anlage which gives rise to the ovary and the vitellarium, the female[1] genital ducts develop. Therefore, although they are strictly speaking somatic structures, their cells will be considered here, with regard to the method of cell division, as derivatives of the female anlage. Their early history, therefore, is the same as that of the ovary and vitellarium. A thickening of nuclei appears median to the longitudinal excretory tubes and gradually extends itself both outward and inward. The outward extension becomes the vagina, seminal receptacle and fertilization canal; the inner not only gives rise to the ovary and vitellarium but also to the remaining part of the oviduct, the vitello duct and the uterus. These various structures are in no sense an invagination from the outside for their development begins in the middle part of their length and the connection with the outside, through the cirrus pouch, is quite secondary to the formation of the lumen of the tube. The lining is, of course, an epithelium; but it is not an invagination from the exterior. That of the cirrus pouch, however, is an invagination and might be looked upon as an ectoderm. But in the other parts of the sex ducts the epithelium is clearly not ectodermic in origin although histologically it resembles the cuticle and is connected with it through the cirrus pouch.

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[1] The development of the male ducts will not be touched upon here.

In their histological structure, the walls of the uterus, oviduct and seminal receptacle are made up of a simple layer of flattened cells probably with a basement membrane. The oviduct furnishes exception to this statement at its opening into the ovary where a layer of circular muscle fibers is demonstrable, and in that part of its course which is known as the fertilization canal. In this latter canal the structure more nearly resembles that of the vagina. The vagina beginning at the lumen consists of ciliary projections, a layer of nuclei embedded in a homogeneous cuticle, longitudinal and circular muscle fibers and a cellular layer.

These canals develop fairly quickly and have all (uterus excepted) finished their growth as far as cell division is concerned long before the sex products call for their use. Upon the passage of the eggs from the ovary they degenerate and their place is taken by the embryo filled uterus.

Whether the ducts grow by more and more parenchymal nuclei becoming involved in the proliferating area, as Child thinks, rather than by the actual outgrowth of the original anlage by the multiplication of its cells is not clear to me. It seems probable that the extension occurs by both methods of growth. The oviduct and the vitello duct are unquestionably formed by the differentiation of part of the cells in the primary anlage for it is in the middle of the anlage that these ducts develop. In the vagina it is not certain that the parenchyma cells whose position it occupies were numerous enough to provide all the cells in its walls without the aid of some outward growth from the region already formed. There is no fundamental difference, however, in the two methods for in either case the cells are of parenchymal origin.

The conditions in the early primary anlage have already been set forth. Mitoses are few, but they do occur. There is no certain evidence for amitosis although many nuclei are arranged in pairs. I have previously given my reasons for the view that amitosis does not occur at this stage of development.

But in the stages beginning with those in which the anlagen of the various organs can be distinguished the method of cell division can be conjectured only. Mitoses are very few, indeed, and occur only in the borders of the proliferating region. On the other hand, the nuclei are small and irregular in size and are frequently closely crowded together in groups of two, three or even more. My preparations do not show actual cases of amitosis, however, although conditions would seem to be quite favorable for its occurrence. Other than the rare mitotic figures which occur there is absolutely no evidence as to the method of nuclear multiplication (see Figs. 51 and 52).

The opinions with regard to the regions in which active cell divisions are occurring expressed by Child ('07*e*) in the following statements do not seem to be well founded according to my observations. He says: "In the early stages there is a marked difference in the size of the nuclei in the central and the peripheral portions of the area in which proliferation is occurring. . . . Evidently proliferation is much more active in the central than in the peripheral regions of the proliferating area. In somewhat later stages the rapidity of division apparently decreases and the nuclei of the central regions gradually increase in size until they are almost or quite as large as those about the periphery. . . . From this stage on the differentiation of the walls of the ducts gradually takes place: muscle fibers develop, a lumen appears, and nuclear divisions become less and less frequent."

In this quotation (the omitted parts have to do only with details not bearing upon the point which I wish to consider) the criterion of proliferation seems to be smallness of nuclei, although in the figures illustrating these statements constricted nuclei, amitoses, are present. As previously stated, these regions in my sections do not show amitoses. But mitoses are to be seen (rarely, it is true) in the borders of the proliferating region. The presence of mitotic figures in this region, it seems to the present writer, is a most significant fact—where but in the borders of a region which is extending its area should division figures be found?—and the small size of the nuclei at the center is of little importance. These central cells are merely beginning differentiation while the proliferation takes place at the periphery of the sex duct anlage.

The method of cell multiplication in the female sex ducts of *Moniezia* cannot at the present time be positively stated. I find no certain evidence for amitosis and that for mitosis is, perhaps, insufficient to account for the growth which has taken place. Nevertheless, I have seen some cases of mitosis here.

Fertilization and Maturation.—The process of fertilization in the eggs of *Moniezia* can be followed only with the greatest difficulty. Although one may examine carefully a great number of proglottids he will rarely find a stage showing the entrance of the sperm and its course prior to the maturation divisions. I am, therefore, unable to amplify the account given by Child ('07*e*) of the early stages of fertilization.

From the ovary the passage through the oviduct to the uterus is so rapid that eggs are seldom found in that part of the duct. Linton ('08) has observed the process of fertilization in a live trematode, and found it to be of very short duration (less than 40 seconds). In *Moniezia* one often finds segments with embryos in the uterus undergoing maturation and eggs in the oviduct ready for fertilization, but the cases are rare in which there are eggs in the fertilization duct. Doubtless here also the passage is very rapid and probably, as Child thinks, periodical.

The eggs pass from the ovary into the uterus through a more or less coiled oviduct. They meet the spermatozoa at the mouth of that branch of the oviduct which comes from the seminal receptacle and are there fertilized. Near this point the vitello duct after passing through the vitellarium and shell gland joins the oviduct—the point of union being known as the oötyp—and this latter duct continues to the uterus.

Cases of polyspermy frequently occur; that is, some eggs show more than one male pronucleus. I have not observed the entrance of several spermatozoa into the same egg, but this is not surprising since the entrance of a single spermatozoön is rarely seen. In many proglottids some of the segmenting eggs ultimately degenerate; possibly they include the cases of polyspermy.

In certain proglottids ova are found in the seminal receptacle, sometimes in quite large numbers. As the seminal receptacle is not ruptured in many of these cases it would seem that in passing through the oötyp the eggs had crowded past the branch of the oviduct leading to the uterus and forced their way through the fertilization canal into the seminal receptacle. There they degenerate. It is, of course, very probable that polyspermy occurs here but so densely packed are the spermatozoa about the egg that the process cannot be followed.

For figures of the fertilization stages as well as for additional figures of the maturation divisions the reader is referred to Child's paper ('07*e*).

Child's account of the maturation divisions agrees in essentials with my observations, and I shall, therefore, merely give a brief résumé of this stage of development.

The entrance of the spermatozoön furnishes the stimulus for the formation of the vitelline membrane and for the beginning of the maturation divisions. The first polar body is usually formed by the time the egg has entered the first loops of the uterus and the second polar body follows soon after. The first division requires more time than the second if the relative frequency in which the two stages occur may be regarded as a criterion. Many more first divisions occur in my material than second.

The characteristics of these divisions are large globular centrosomes—ring-shaped in section—faint astral radiations,[1] and a very long spindle on which small chromosomes are to be seen distinctly. An equatorial plate is not usually formed as the chromosomes pass to the poles irregularly. Figs. 53 and 54 illustrate these facts.

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[1] Child stated that he was unable to see asters but thought they probably occur. I have succeeded in distinguishing faint radiations in numerous maturation stages.

The reconstruction of the female pronucleus is shown in Fig. 55. These stages suggest amitotic division of the ovum—some more than that figured; their true nature, however, is readily ascertained.

I am unable to see that the maturation of *Moniezia* has any bearing whatever on the "hypothesis of individuality" of chromosomes, which Child says these mitoses do not appear to strengthen, for it does not seem to me that evidence either pro or con is presented here. Indeed, the whole question involved is not so much whether the chromosomes maintain their individuality as it is whether the mechanism of division will give an exact sorting of the male and female elements in the germ cells or a mere separation of unequal parts of the nuclei. If mitosis could be established as the universal method of cell division that fact alone would by no means warrant the assumption of chromosome continuity, as Child would seem to imply that it would do. Among those who believe that mitosis will be found general in germ cells are many who do not accept the individuality hypothesis as it is now put forward. Furthermore, proof of that hypothesis will demand observations upon a more favorable object than *Moniezia*. I would emphasize the fact, therefore, that *Moniezia* presents no evidence upon the individuality hypothesis.

Cleavage.—The steps of the cleavage process in *Moniezia* were discussed in 1881 by Moniez from an embryological point of view, and by Child in 1907 from the point of view of cell division. The results of Moniez are very suggestive and deserve to be repeated in the light of more recent discoveries on cleavage and with later methods of technique. Since the cleavage of *Moniezia* is deserving of this broader treatment I shall not here attempt other than the most general statement of the process but shall concern myself with the method by which segmentation is accomplished.

The type of cleavage, as will be seen from a glance at the figures, from 56 on, is that of a large macromere giving off small micromeres at the animal pole.[1] The later divisions bring about the formation of a morula which gives off two layers by delamination and then develops into the hexicanth embryo. This cleavage especially in its earlier course differs markedly from that in *Tænia serrata* (cf. Janicki's figures with mine).

As cleavage proceeds many embryos become elongated, a condition which Child suggests is due to pressure of the uterine walls. This, of course, does not represent the future axis of elongation. That Child's suggestion is probably the correct one is shown by Moniez's figures from entire embryos which are all spherical and do not include any elongated stages.

During the early part of my study while I was working upon material in which the results of fixation were good but by no means perfect I thought I had found the syncytial condition of which Child speaks: "In most cases a number of nuclear divisions occur before cell boundaries become visible in the egg. . . . As cleavage proceeds the egg is gradually divided into blastomeres containing yolk and blastomeres without yolk. In earlier stages the yolk bearing blastomeres often contain two or more nuclei, but in the later stages after cytoplasmic cleavage is more advanced they usually contain one relatively large nucleus. In other words as these yolk bearing blastomeres are gradually reduced in size by successive cleavages the cytoplasmic cleavages keep pace more nearly with the nuclear divisions. In the yolkless portion of the egg, however, nuclear division continues far in advance of cytoplasmic division as far as the cleavage has been followed; the consequence is that each blastomere contains several or many nuclei of relatively small size."

Later, in studying material in which fixation had been more nearly perfect, I saw at once that the supposed syncytial condition was in reality an artifact. From the very first the micromeres can be distinguished as such, an observation corresponding with the figures of Moniez made about thirty years ago. The cytoplasmic membrane becomes completely constricted even before the telophase is finished as in Fig. 57. In properly fixed material I have never seen an egg syncytium. The work of

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[1] Assuming that, as in all known cases except in the ctenophores according to Korschelt and Heider, the polar bodies indicate the animal pole.

Moniez contains much which a reinvestigation with modern methods of technique will substantiate.

As regards the method by which segmenting eggs divide, the following sentences are illustrative of Child's position:

"But although the first cleavage is usually or always mitotic, there can be no doubt that amitotic division appears very early in the course of cleavage.

"Cases of mitosis are rarely seen after the first cleavage but amitosis is of frequent occurrence.

"Rarely a case of mitosis is observed: in all the hundreds of eggs in cleavage stages which have been examined, not more than a dozen cases of mitosis have been seen in stages later than the first cleavage. When mitosis occurs it apparently always involves one of the larger nuclei. Mitotic divisions of the small nuclei in stages like those shown in Plate VI. have never been observed. The smaller nuclei are, without doubt, dividing more rapidly than the larger, and we are probably justified in concluding that amitosis occurs in those regions of the egg where division is most rapid, while mitosis is found, when it occurs at all, among the nuclei which are dividing more slowly."

With the first of these statements only can I agree. Figs. 56 to 63 show the successive steps in the cleavage process up to the eight cell stage and every division is by mitosis. Fig. 64 shows several cells from an embryo of thirty cells, among which are two blastomeres in mitosis. Fig. 65 is drawn from a single blastomere in an embryo of from fifty-five to sixty cells; it shows an early phase of mitosis. These stages are not rare or difficult to find in my material. I have examined segmenting eggs of numerous other animals and do not hesitate to say that the mitotic figures in *Moniezia* are as frequent as in other lots of eggs selected at random.[1] Indeed, in five sections cut four micra in thickness, in which the uterus did not contain a relatively large number of eggs over twenty cases of cleavage mitoses which were deemed worthy of sketching were found. In another case, in 125 embryos (all that were present in the given region) there were 28 cleavage mitoses; in another of 211 embryos were 53 mitoses; in still another, 124 embryos showed 40 mitoses. The evidence that these cleavages occur by mitosis is of the most positive and convincing character. Cleavage mitoses are more abundant in my material than the second maturation division which is, of course, mitotic.

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[1] Of course in those cases where artificial fertilization is practical the eggs are selected in the desired stage; such cases may not fairly be compared statistically with the one under discussion.

Furthermore, I have seen only one condition which could possibly be interpreted as amitosis; it is shown in Fig. 59. This figure represents the stage following the telophase of the division cutting off the third micromere. The same condition obtains following other telophases, however. The nucleus after the telophase at first takes on an irregular appearance but later becomes rounded out into the typical shape. This stage is exactly comparable to the reconstruction stage of the female pronuculeus after maturation. It is to be interpreted as the final phase of a mitosis rather than the precursor of an amitotic division.

In the later cleavages, although I have not as yet followed out all of the cell lineage, I have never found any reason to doubt that mitosis is the method of cell division. Mitosis certainly occurs frequently and I have seen no evidence of amitosis. The two figures given are of typical cases; further illustrations would be mere reduplication of the evidence.

In the smaller blastomeres I have never seen any sign of direct division; on the other hand, Figs. 60 and 62 show cases of mitosis in these cells.

To the present writer the facts in the cleavage of *Moniezia* seem to admit of no other interpretation than that mitosis is the method by which segmentation is accomplished.

Summary of the Evidence.

This summary will include the evidence gained from the study of the female sex products from the time of their appearance as the primary anlage of the ovary to the completion of segmentation. It will not include the vitellarium of sex ducts since the evidence given by them is not thought sufficient to be conclusive in favor of either view.

It has been stated that the cells composing the primary anlage might arise *de novo*, by migration, by amitosis and by mitosis. The first of these possibilities was dismissed as highly improbable; the burden of proof is upon him who upholds this view. The second method of origin was admitted to be possible, but it was thought to play only an unimportant rôle, if any at all, in the development; and even if the assumption of migration as a factor—positive proof would scarcely be procurable—were found to be necessary it would merely throw the question of division back into the earlier stages. It was held, therefore, that the cells under discussion must arise *in situ* by direct or indirect division. It is the purpose of this summary to present the evidence for the two views as concisely as may be.

It has been shown, furthermore, that at two periods of development only may active growth by cell division be looked for: namely, during the period of pre-oögonial and oögonial divisions and during the cleavage of the embryo; the rest of the growth involved is that of increase in size and in differentiation. The discussion, therefore, limits itself to the question: is amitosis or mitosis the method by which the pre-oögonial and oögonial and the cleavage divisions occur?

The evidence, as I have found it, favoring the view that amitosis occurs during the periods of development mentioned is as follows:

First, there are in the development of the oögonia fewer cases of mitosis to be found than one would *a priori* expect, and it might be claimed that the number present is not sufficient to account for the cell multiplication which must have occurred.

Second, the arrangement of the nuclei in pairs suggests in the light of the fact just stated that the cells may have arisen by direct division. Two nuclei often occur close together but separated more or less from their neighbors.

Third, constricted and indented nuclei do occur in the early stages of development of the oögonia; but they occur in my material only in cases of imperfect fixation, so they lend very little support to the amitosis view.

Fourth, in the cleavage stages but one condition (unless accessory sperm nuclei might be confused with direct cell division) has been seen in properly fixed material which might be interpreted as favoring the amitosis view: the condition represented in Fig. 59.

The evidence opposed to amitosis and favoring the view that mitosis is the method by which divisions occur is as follows:

First, mitotic figures do occur throughout all stages of the development of the tissues considered, and they occur in the peripheral (or growing) regions of the organs. Analogy may be drawn from cases like *Planorbis* and *Ascaris* in which the oögonial divisions are very difficult to find, and yet are known to occur regularly.

Second, stages of nuclear constriction are not found in sufficient number to account for the arrangement of the nuclei in pairs if amitosis be the method by which they arise. It is logical to expect that steps in the process would be found if the arrangement in pairs is the end result of amitotic division. As has been repeatedly stated, it is not possible to establish a complete series of stages in the auto-constriction of nucleus and cell body. If periodicity is a factor in cell division (see below) the arrangement may be looked upon as favoring the mitosis view. Finally, it is most probable, although impossible to prove, for the preoögonia and oögonia are not favorable for such study, that there may be some movements of the halves toward each other analogous to the movements of telokinesis in other tissues, for in all cases carefully studied with regard to this point, including developing eggs, epithelial tissues, etc., such movements have been found. I have myself found evidences of them (not shown in the present series of figures) in the cleavage of *Moniezia*.

Third, the occasional cases of constriction and indentation which occur have not been shown to be normal steps in division.

Fourth, the condition represented in Fig. 59 is, as previously stated, the final stage of mitotic division rather than the beginning of amitosis.

Fifth, the evidence favors periodicity as a factor in mitotic divisions. (See below, under discussion.)

Sixth, that the maturation and first cleavage alone should occur by mitosis as described by Child is difficult to reconcile with amitotic divisions elsewhere throughout the series.

Seventh, the evidence that the cleavage divisions occur by mitosis is of the most positive character. It cannot be too strongly emphasized.

Eighth, I have after diligent search upon carefully prepared material been unable to establish a series of stages in the autoconstriction and subsequent division of the nucleus and cell body by amitosis.

Considering the evidence as set forth, it seems to the writer that one is forced to the conclusion that mitosis is the method by which pre-oögonial, oögonial and cleavage divisions are accomplished. These are the divisions to which the chief interest attaches.

Discussion.

Since the account of Child appeared describing amitosis in *Moniezia* and other forms there have been a number of *a priori* suggestions offered by various workers as possible explanations of the conditions which he describes. It will be of interest to examine some of these suggestions in the light of the facts which I have observed in *Moniezia.*

Cary on the basis of his observations on *Diplodiscus* suggests that the telophase of an intranuclear mitosis would give all the appearances of true amitoses. I have carefully searched for evidences of intranuclear mitoses in *Moniezia* but have found none. I have seen cases which at first resembled a faint intranuclear spindle, but upon more careful study it became clear that they were merely chance arrangements of linin threads and chromatin granules and not mitosis. Furthermore, as already shown, distinct cases of mitosis of the regular type are not difficult to find. It is, of course, true that the condition which Cary describes would resemble amitosis, but I have not found it present in *Moniezia.*

Boveri calls attention to the fact that figures similar to those found in *Amblystoma* (Child, 07*a*) are also found in *Triton.* In this latter form Rubaschin found that after mitosis the nuclear material does not fuse to form a single vesicle in the blastomeres but two are always formed. They never separate but go through mitosis together. In *Moniezia* there is no evidence of this kind of phenomenon.

As there is no "Nebendotter" in the eggs of *Moniezia* my former suggestion that that structure might give a misleading appearance of amitosis is not borne out.

As polyspermy occurs here accessory sperm nuclei might be wrongly interpreted as amitosis. The condition, however, is not difficult to distinguish.

Cell migration has already been discussed in detail as a method of origin for the pre-oögonia. As stated before there is not much evidence that it occurs. It certainly is not the exclusive factor and probably not even an important one.

There yet remains my suggestion that mitosis may be of short duration and occur in waves or at more or less definite periods depending upon some unknown physiological factor—an interpetation which Child refuses to accept. He says ('10, p. 113): "Not only the cells of the neck region but the cells of the scolex, which does not take part in the growth of other regions are undergoing mitosis in this specimen.[1] Moreover, I am as yet unable to convince myself that the many cases of apparent amitosis which I have observed in the neck region of other individuals, are errors of observation, or something else than nuclear division." Nevertheless, is not the position that "the relative frequency of mitosis and amitosis in certain species and even in single individuals may vary greatly according to conditions" in its practical application an admission of my suggestion?

This suggestion is based first upon certain *a priori* considerations. Beckwith described cleavage mitoses in *Pennaria* and *Clava* occurring from 4 to 6 A.M. only; in certain insects and many plants mitosis occurs at night only; in onion root-tips there are two periods of mitotic activity daily, at 1 P.M. and at 11 P.M. and if the specimens are collected at any other times than these there will be found a paucity of division figures. I have seen grass root-tips in which not a mitotic figure could be seen although young cells of the proper age for multiplication were abundant. Amitosis has never been suggested for such cases as this. Secondly, the suggestion has support in certain observational evidence not the least of which is Child's young worm in which almost every nucleus is in mitotic division. The fact that I am unable to convince myself that amitosis occurs and the fact that mitosis does occur in some specimens in greater abundance than in others (this applies to *Tænia* as well as to *Moniezia*) and even some organ systems show more figures than others (I have one specimen in which the majority of the testes cells are in mitosis) point strongly to periodicity as a factor in growth. The arrangement of nuclei in pairs may be regarded as a point in favor of this view. That the scolex of this worm should be in division is of no significance for even if it takes no part with the neck region in the formation of new proglottids, and this is not demonstrated, its own growth must be accounted for. The scolex of a young worm is smaller than that of an older one.

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[1] A young specimen, found subsequent to his earlier accounts, consisting of scolex, neck region and a few of the youngest proglottids in which almost every nucleus is undergoing mitosis.

Furthermore, if it can be shown that the eggs pass through any one stage periodically it becomes all the more probable that they also pass through others periodically. The evidence is strong that the eggs undergo maturation in this manner, and that fact is most easily explained by periodicity of oögonial divisions.

Child holds that mitoses decrease in frequency with increasing age. Let me suggest that perhaps that factor, whatever it may be, that operates to free the intestine of adult sheep from tapeworms may also operate to check mitotic division during the latter part of the period in which the worms can yet live there.

The evidence, I believe for the various reasons given, strongly favors periodic mitoses as an important factor in the growth of the cestode, *Moniezia*.

Comparison of *Tænia* and *Moniezia*.

Throughout the course of this discussion it has been made clear that *Moniezia* differs from *Taenia serrata* in numerous details, both anatomical and cytological. Yet it is also clear that the differences, while often striking, are differences not of physiological activites and principles but of structural features and structural differentiation.

Most striking of all the differences is that of gross structure as seen in the female reproductive apparatus. Such wide variations from a common type are not usually found within a single family of animals. In both the uterus is single[1] and median but the other organs are arranged in pairs, there being two ovaries and two sets of auxiliary glands and ducts as well as two genital pores in each proglottid in *Moniezia* while in *Tænia* the ovaries are double but they discharge into a common duct and the other parts of the reproductive organs are single.

But the most interesting comparison of the two forms for present purposes is that regarding cytological characteristics. In both the anlagen of the organs arises as a thickening of the parenchyma cells and the period of cell proliferation is strictly comparable on the two. In *Tænia*, however, it is of less duration as seen by the fact that it extends over fewer proglottids. The method of yolk formation is dissimilar in appearance only, for if the yolk granules of *Moniezia* were to accumulate on one side of the nucleus and fuse the condition would be exactly that of *Tænia*. I have not discovered that there is any difference in the principles of division operative in the two forms.

Between the types of cleavage and early development of the embryos of the two forms there are some noteworthy differences but as they are embryological in character they may not properly be included here. It will be seen from this comparison that there are sufficient differences between *Tænia* and *Moniezia*, although they are members of one family, to justify the claim that observations on the former cannot be used *a priori* as evidence against the latter; but that actual study does not disclose any real differences in the principles of growth and development in the two cases.

CONCLUSIONS.

In conclusion, the evidence presented in this investigation goes to show: (1) that it cannot now be positively affirmed whether mitosis or amitosis is the method which obtains in the development of the vitellarium and female genital ducts of *Moniezia;* (2) that in the early stages of sex cell development mitosis unquestionably occurs (probably periodically), while amitosis is not evident in my preparations; and finally, (3) that there cannot be the slightest doubt that the cleavage of the ovum takes place by mitosis.

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[1] Stiles and Hassal state that the uterus of *Moniezia* is ontogenetically double.

Note.

While this paper was in press an article by Gough ["A Monograph of the Tapeworm of the Subfamily Avitellininæ, being a revision of the Genus *Stilesia*, and an account of the Histology of *Avitellina centripunctata* (Riv.)"] appeared in the *Quarterly Journal of Microscopical Science* for February, 1911, wherein he describes the development of the eggs of *Avitellina*. He, too, finds Zenker's fluid best for cestodes. As in *Moniezia*, yolk cells do not become attached to the eggs to form compound structures. The large mass which he calls chromatin (Figs. 57–63) apparently correspond to my nucleoli (my Fig. 30); there can be no doubt that in *Moniezia* these are nucleoli and that no other nucleolar structures occur, as shown by differential staining. The extrusion into the cytoplasm of chromatin which breaks up to form the yolk nucleus (see p. 373) has not been observed in *Moniezia*. I would suggest that his Fig. 63 perhaps corresponds to my Fig. 26 which is a synapsis stage not merely the first step in a mitosis. Gough speaks of the maturation mitoses as occurring very rapidly. As *Stilesia*, and, therefore, *Avitellina* is quite closely related to *Moniezia* the comparison of the two is of interest, particularly with regard to the fact that compound eggs do not occur in either

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EXPLANATION OF PLATE I.

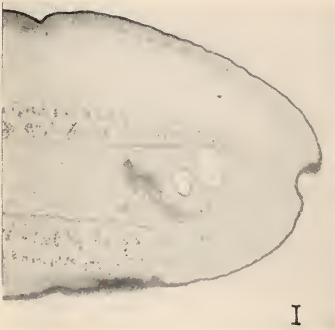
Photomicrographs were made with a Leitz no. 3 objective and no. 2 ocular except IV. in which a no. 1 ocular was used. All figures were drawn with a Zeiss compensating ocular no. 12 and a 1.5 mm. objective at table level with the aid of a camera lucida, giving a magnification of about 3,500 diameters.

PHOTO. I. End of a section through a young proglottid, showing the primary anlage of the female reproductive organs.

PHOTO. II. Primary anlage differentiated into the anlagen of the ovary, vitellarium and oviduct.

PHOTO. III. Later stage showing finger-like processes of the ovary. Dorsal to the middle of the ovary is the tip of the vitellarium.

PHOTO. IV. Stage of maximum development. *exc*, excretory canal; *od*, oviduct; *v.t*, vitellarium; *vt.d*, vitello duct; *ov*, ovary; *ut*, uterus; *t*, testes; *f.d*, fertilization duct; *s.r*, seminal receptacle.



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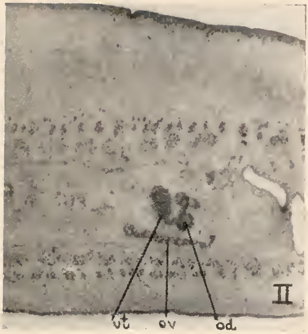

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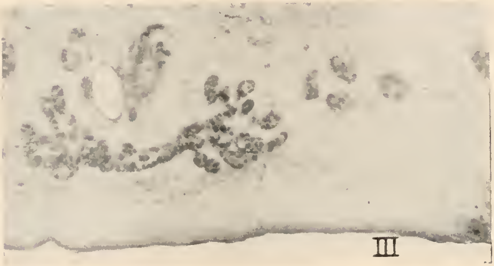

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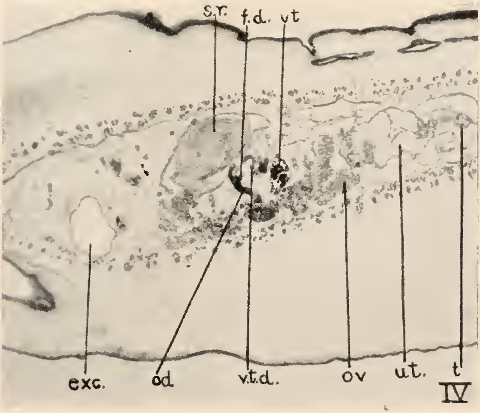

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EXPLANATION OF PLATE II.

FIGS. 1 and 2. Parenchyma cells for comparison with the cells of the primary anlage; from young proglottids. Fig. 2 illustrates the arrangement of the cells in pairs common in all undifferentiated tissue of the cestode. Cytoplasmic boundaries are difficult to make out in such cells as these in all but the best fixed specimens.

FIGS. 3–5. From primary anlage in same section as Fig. 2. Fig. 3 is from the inner growing end of the anlage, to be the ovary; Fig. 4 is from the middle region, to develop into the vitellarium, etc.; Fig. 5 is from the outer growing tip, to form the vagina. Hermann, iron hæmatoxylin.

FIGS. 6 and 7. From the primary anlage of the same section from which Fig. 1 was taken; Fig. 6 from the middle, Fig. 7 from the outer end. Zenker, Ehrlich-Biondi.

FIG. 8. A case of mitosis in the primary anlage. King, iron hæmatoxylin.

FIG. 9. Mitosis in a pre-oögonium. Zenker, safranin and gentian violet.

FIG. 10. Polar view of a mitosis from the same anlage as Fig. 9.

FIG. 11. Cell from near the growing tip of the ovary anlage; a pre-oögonium. Zenker, Ehrlich-Biondi.

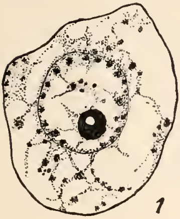
FIG. 12. Group of pre-oögonia just before the formation of the follicular membrane. Cytoplasmic boundaries are not well preserved in all cases. The overlapping of such nuclei as are shown here gives a misleading suggestion of amitosis. King, iron hæmatoxylin.

FIG. 13. A stage in the mitosis of a late pre-oögonium before the condensation of the chromosomes. Hermann, iron hæmatoxylin.

FIG. 14. The most distinct case of mitosis found throughout the oögenesis; spindle fiber bundles are very strong. A late pre-oögonium. King, Ehrlich-Biondi.

FIG. 15. Pre-oögonium, slightly later than Fig. 12. King, iron hæmatoxylin.

FIG. 16. A young oögonium in mitosis; polar view. Zenker, iron hæmatoxylin.



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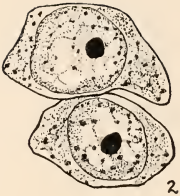

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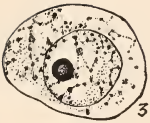

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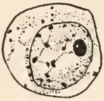

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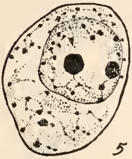

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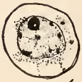

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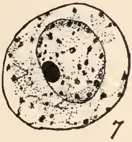

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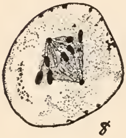

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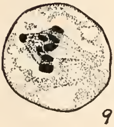

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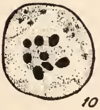

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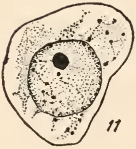

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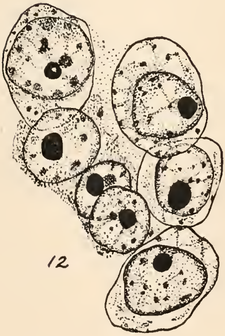

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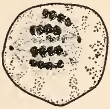

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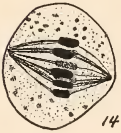

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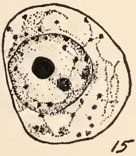

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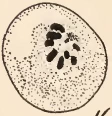


16

A. RICHARDS

Explanation of Plate III.

Fig. 17. Young oögonia, showing three sets of nuclei arranged in pairs. Only one nucleolus is present in each, the large black granular masses being chromatin. Cytoplasmic boundaries not well preserved. King, iron hæmatoxylin.

Fig. 18. Oögonia; the upper nucleus stained very heavily. King, iron hæmatoxylin.

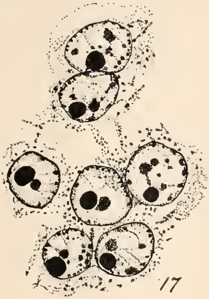
Fig. 19. An oögonium beginning growth. King, iron hæmatoxylin and Bismarck Brown.

Fig. 20. Mitosis in an oögonium just before the growth period begins. King, iron hæmatoxylin.

Fig. 21. An oögonium beginning growth; the chromatin is beginning to condense in preparation for the synapsis stage. King, iron hæmatoxylin.

Fig. 22. Oögonium from the same ovary, slightly later in development.

Figs. 23–28. Progressive stages of synapsis, 23–26 from the same slide. King, iron hæmatoxylin. 27, King, iron hæmatoxylin. 28, King, Ehrlich-Biondi. In Fig. 26 are to be seen the beginnings of the yolk nuclei. They do not regularly appear at this stage.



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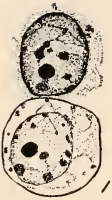

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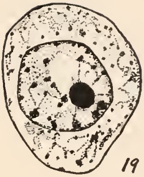

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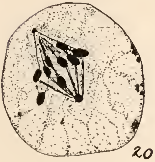

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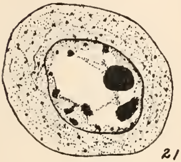

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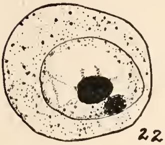

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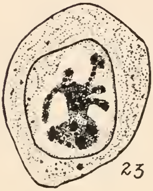

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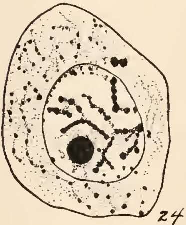

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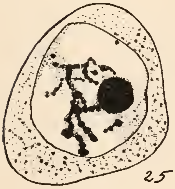

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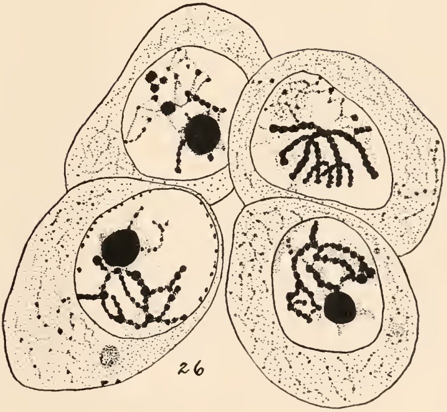


26

A RICHARDS

Explanation of Plate IV.

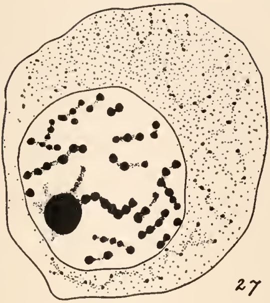
In the latter part of the growth period, following synapsis, yolk is produced (Figs. 31–35) and the nucleoli exhibit certain characteristic features. Figs. 28, 29 and 30, selected from the oöcytes, show a nucleolus proper, paranucleolus, endonucleolus and certain peculiar dark (chromatic?) bodies. These bodies give the characteristic staining reaction of chromatin, very dark with gentian violet. The nucleolus with the triple stain is violet, paranucleolus pink, endonucleolus dark. In the paranucleolus a reticulum supporting the endonucleolus can often be seen (29g).

Fig. 29. King, Ehrlich-Biondi.

Fig. 30. King, triple.

Fig. 31. Oöcyte showing yolk nucleus. The nucleus is not well fixed in this material. Chrom-oxalic, iron hæmatoxylin.

Fig. 32. Sections above the nucleus, passing through the yolk nuclei. Chrom-oxalic, iron hæmatoxylin.



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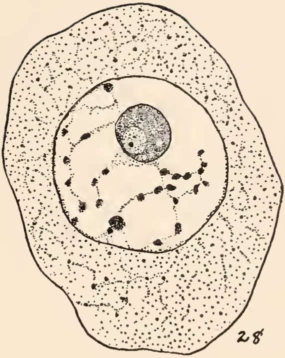

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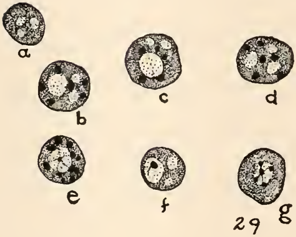

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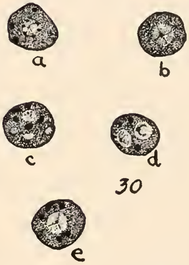

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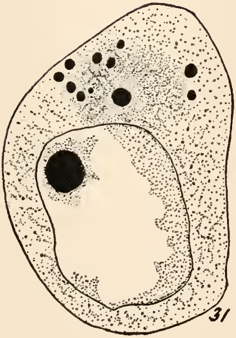

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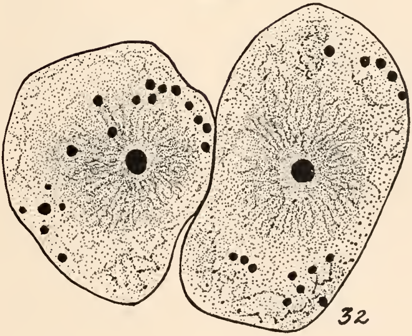


32

A. RICHARDS

EXPLANATION OF PLATE V.

FIGS. 33–35. First oöcytes, the ovarian eggs. Yolk globules shown in black in Fig. 35 and indicated by dotted circles in the other figures; chromatin in the characteristic arrangement. Two peculiar black bodies in Figs. 34 and 35 may perhaps be centrosomes or spheres. Fig. 33, King, iron hæmatoxylin; 34, Zenker, Ehrlich-Biondi; 35, King, iron hæmatoxylin.

FIGS. 36–50. Development of the vitellarium. Character of the early development is shown in Figs. 4 and 6 from the primary anlage.

FIG. 36. Corresponds to Fig. 11 in time of development. Zenker, Ehrlich-Biondi.

FIG. 37. Same stage as Fig. 12. King, iron hæmatoxylin.

FIG. 38. Growing vitellarium cell from some section as Fig. 15. King, iron hæmatoxylin.

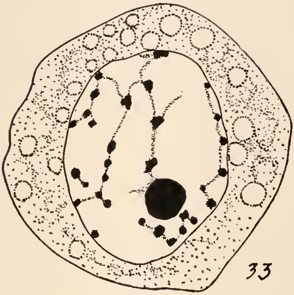
FIG. 39. From the medullary portion of the anlage, the shell gland from the same section as Fig. 37. Cytoplasm drawn out into fibers.

FIG. 40. Shell gland region; same slide as 41 and 42.

FIGS. 41 and 42. Vitellarium cells; both cells simulate mitosis due to the heavy linin strands across their centers. Fig. 42 was at first taken for a case of "endogenous division." King, iron hæmatoxylin.

FIG. 43. From growing vitellarium, about the same stage as Fig. 20, a characteristic mitosis. King, iron hæmatoxylin.

FIG. 44. Just before the formation of the yolk body. The dense cytoplasmic mass is probably a yolk nucleus. King, iron hæmatoxylin and Bismarck Brown.



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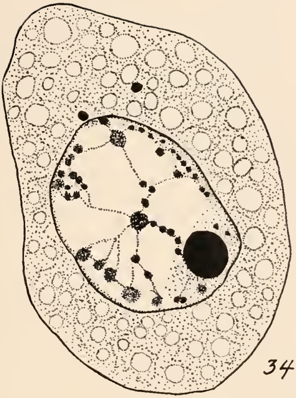

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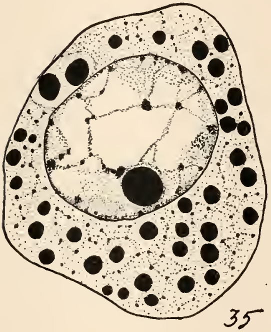

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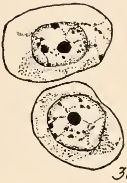

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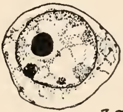

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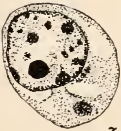

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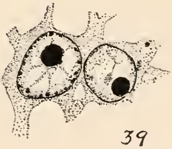

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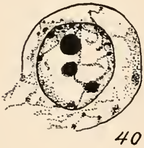

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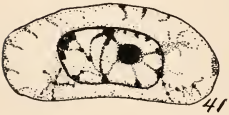

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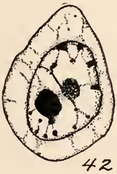

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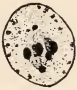

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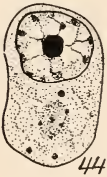


44

A. RICHARDS

Explanation of Plate VI.

Figs. 45–49. Stages in the development of the yolk body in the vitellarium cells. King, Conklin's stain.

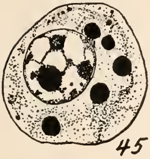
Fig. 50. The appearance of a much decolorized yolk mass. *b* is the same yolk mass shown in *a* at a lower level; probably the central bodies here are remnants of the yolk nucleus. King, iron hæmatoxylin.

Fig. 51. Cells early in the development of the female genital ducts. Cytoplasmic boundaries are not well fixed in this preparation. The nuclei show no evidences of division either direct or indirect. Arrow points in the direction of the axis of the duct. King, iron hæmatoxylin.

Fig. 52. A cross section from a somewhat later stage in oviduct formation. A case of mitosis is shown here. Formol-sublimate, iron hæmatoxylin.

Fig. 53. First maturation division of an oöcyte. King, iron hæmatoxlyin.

Fig. 54. Second maturation division. Zenker, iron haematoxylin.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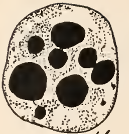
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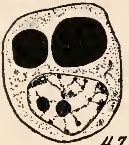
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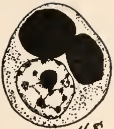
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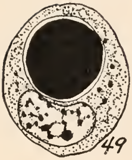
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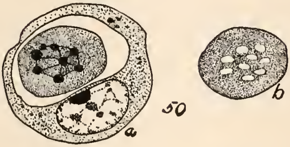
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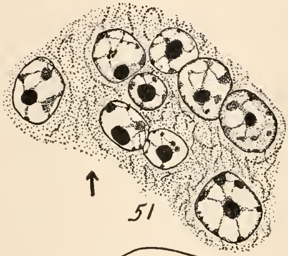
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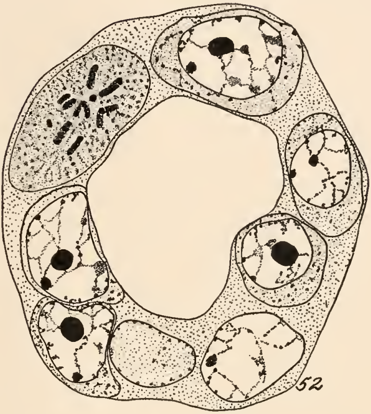
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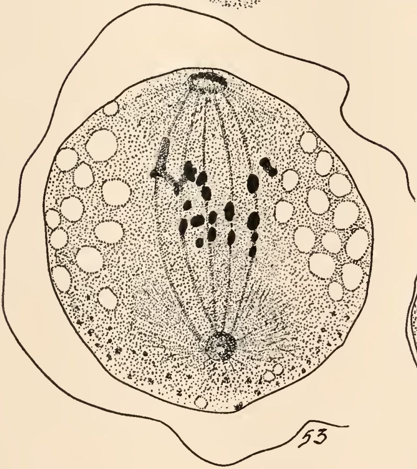
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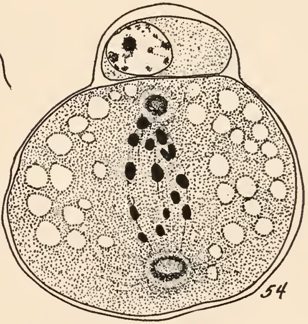

54

A RICHARDS

EXPLANATION OF PLATE VII.

FIG. 55. Reconstruction of the female pronucleus; male pronucleus near. The other polar body in the next section. Zenker, Conklin's stain.

In the remaining illustrations the blastomeres not shown in the figure were to be found in the next section.

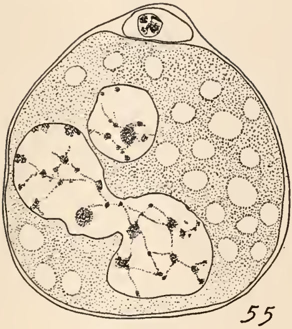
FIG. 56. First cleavage division. Zenker, safranin and gentian violet.

FIG. 57. Division cutting off the second micromere. Three-cell stage. Zenker, safranin and gentian violet.

FIG. 58. Formation of the third micromere. Dotted line indicates the position of the second micromere. Zenker, safranin and gentian violet.

FIG. 59. Four-cell stage, showing reconstruction of macromere nucleus after the last mitosis. Zenker, safranin and gentian violet.

FIG. 60. Formation of the fourth micromere. Zenker, safranin and gentian violet.



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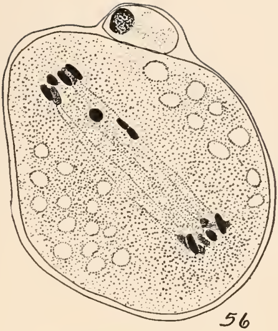

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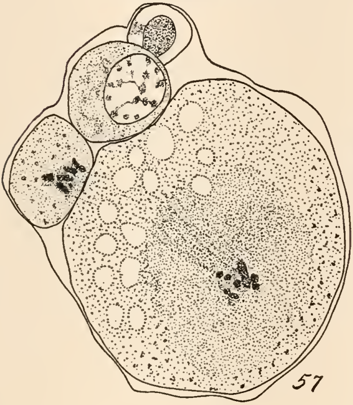

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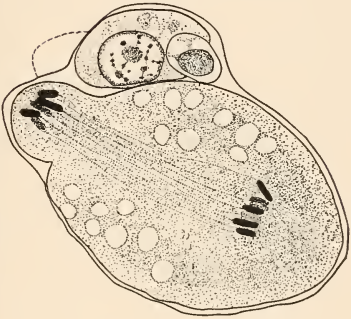

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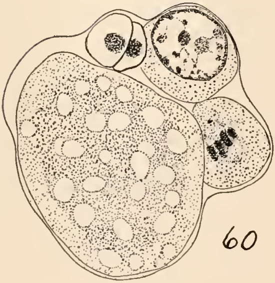

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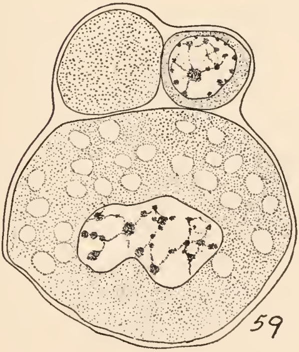


59

A. RICHARDS

Explanation of Plate VIII.

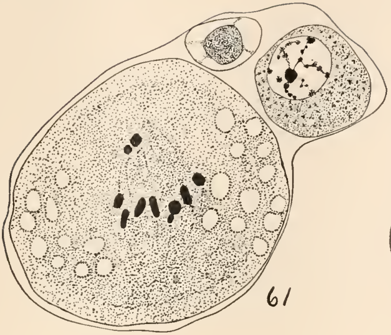
Fig. 61. Six cell stage. Next section shows seven chromosomes belonging to this spindle.

Fig. 62. Seven cell stage. Formol-sublimate, safranin and gentian violet.

Fig. 63. Division in the eight cell stage, only one end of the spindle shown in this section. Zenker, safranin and gentian violet.

Fig. 64. From an embryo of about 30 cells, showing two mitoses. Zenker, safranin and gentian violet.

Fig. 65. A blastomere beginning mitosis from an embryo of 55 to 60 cells. Zenker, safranin and gentian violet.



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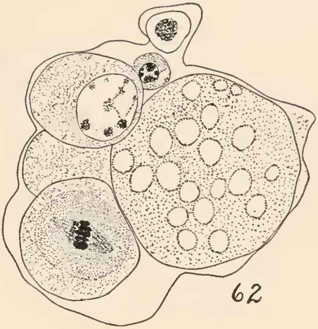

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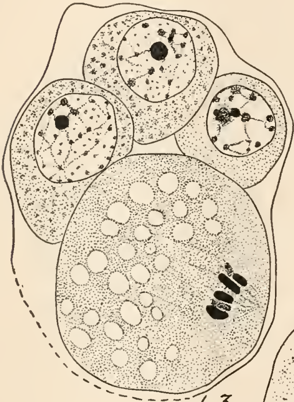

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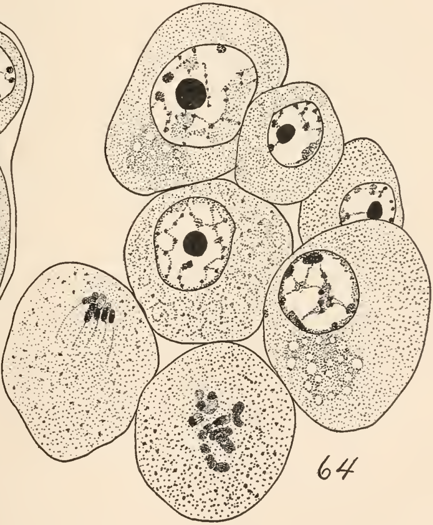

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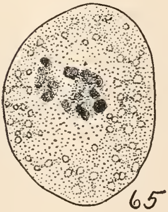

65

A. RICHARDS

A NOTE ON THE METAMORPHOSIS OF THE MUSSEL LAMPSILIS LÆVISSIMUS.[1]

ROBERT E. COKER AND THADDEUS SURBER.

While making a collection of the glochidia of various species of mussels used in this laboratory certain observations have been made which prompt the publication of a preliminary note on the peculiar type of glochidium seen in *Lampsilis lævissimus* and other species, and the metamorphosis during the period of parasitism.

1. Type of Glochidium and Possible Significance.

The glochidium of *Lampsilis alatus* has been observed by Lefevre and Curtis,[2] who describe it as an "axe-head" glochidium. "This possesses hooks which are not homologous with those of the anodonta type and is to be regarded as more nearly related to the hookless forms, an interpretation which is borne out by the fact that the 'axe-head' can be readily imagined as a modification of the glochidial outline seen in some species of *Lampsilis*, which, like *subrostratus*, show an approach to a rectangular outline."

This glochidium (Figs. 3 and 3*a*) is certainly of a very specialized form. In our laboratory we have observed an almost exactly similar glochidium in *L. capax* (Figs. 4 and 4*a*), while in *L. lævissimus* (Figs. 1 and 1*a*) the same shape is observed but the hooks are wanting. It is remarkable that a larva of so specialized a form should be found in two species such as *alatus* and *capax*, the adult forms of which are so extremely opposed in general shape; *alatus* is one of the most compressed forms in the genus, while *capax* is the most inflated (Figs. 4*b* and 4*c*).

L. lævissimus (Figs. 1*b* and 1*c*) and *L. gracilis* are forms suggestive of *alatus* in the compressed character of the shell. They are somewhat more compressed than *alatus* and are much thinner shelled. These two species seem almost to intergrade (in form of shell) so that one sometimes hesitates in the identification of a specimen of intermediate form. On the other hand, the glochidia of these two species show a striking contrast. The glochidium of *gracilis* (Figs. 2 and 2*a*) is oval-pear-shaped and very similar to that of *ligamentinus* or *ventricosus*, or to what we generally regard as a typical form; while that of *lævissimus* (Figs. 1 and 1*a*) is of the "axe-head" type. Comparison of Figs. 1*a* and 2*a* may suggest, however, that the two species are not so dissimilar as at first appears, and *lævissimus* may be thought of as intermediate between *alatus* and *gracilis*.

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[1] Published by permission of the U. S. Commissioner of Fish and Fisheries. Read by title before the American Society of Zoölogists, December, 1910.

[2] "Reproduction and Parasitism in the Unionidæ," *Jour. Exp. Zoöl.*, Vol. IX., No. 1, p. 94.

The remarkable fact, yet, is that *capax* should have this type of glochidium. *Capax* has always been grouped with *ventricosus* and *ovatus*, but *ventricosus*, at least, has a glochidium of the usual form. Whether the greater significance be attached to resemblances in larval form or in adult form, it is a suggestive revelation of the adaptability of fresh-water mussels and the inadequacy of superficial diagnosis. Undoubtedly in the classification of fresh-water mussels an entirely undue stress has been laid upon characters of a superficial nature. The ultimate system will be based on a much more thorough analysis of the actual anatomy of mussels in young and adult stages than has ever been attempted in systematic work on this group. We have not yet a sufficient knowledge of the comparative anatomy of glochidia to judge of their relative value in the study of relationships, but certain considerations derived from the present case are sufficiently striking to be recorded for their suggestive value.

Ventricosus is certainly closely related to *ligamentinus* and *luteolus*, as judged by the external form. The glochidia, too, are much like those of *ligamentinus*. The adult form is specialized most noticeably in being very inflated. *Gracilis* is specialized in a different way, being exceedingly compressed laterally and having compressed teeth; *lævissimus* (Figs. 1*b* and 1*c*) is a more extreme form of the *gracilis* type, the teeth are more blade-like, while the shell is equally compressed from side to side and has well-developed wings. The glochidium, while suggestive of *gracilis* (which is of the "typical" form), is of the "axe-head" form. The adult *alatus* is of somewhat the same compressed form, broadly winged, and with "axe-head" glochidia still further modified by the development of hooks. *Capax* has the same type of glochidium, but what of the adult form (Figs. 4*b* and 4*c*)?

In external shape *capax* and *ventricosus* are so closely alike that it has sometimes been doubted if the two species were properly separable. However, the species *capax* is now universally accepted, for the reason that *capax* is clearly distinguishable from *ventricosus* in: (1) the polished almost rayless character of its epidermis, (2) the compressed form of the teeth, and (3) in the relative thinness of its shell, which is inclined to pinkness in color of nacre. *Now these*, it is significant to remark, *are all characters of lævissimus*. In fact, (1) above is the most certain means of distinguishing *lævissimus* from *gracilis*.

If, then, we were to draw an inference from the glochidia as to a relationship between *lævissimus*, *alatus* and *capax*, there would be strong corroborative evidence in the adult characters, in spite of the fact that *lævissimus* and *capax* are the two extremes in degree of inflation. The similar degree of inflation of *capax* and *ventricosus* would offer only a striking instance of convergence in one character.

2. Change of Form during Parasitism.

The change undergone by *Lampsilis lævissimus* during parasitism is most striking. As a general rule, it has been held that there is practically no growth in size during the period of parasitism—simply a metamorphosis from glochidium to young mussel in rudimentary form. Our observations show a notable exception to this general rule.

While examining the gills of a specimen of the sheep's-head, *Aplodinotus grunniens*, several specimens of encysted glochidia were found in an advanced stage of development. The infection occurred in nature, so that the age of the mussels cannot be stated.

Figs. 5, 6 and 7 show not only the striking change in form, but the enormous increase in size as well. Fig. 5 shows a specimen in side view; the "axe-head" glochidium shell is evident, but the mussel is now nearly circular in outline and several times larger than the glochidium. The specimen shows considerable inflation. Fig. 7 shows the dorsal aspect of a more advanced specimen; the degree of inflation is apparent; the glochidial shell is insignificant in size as compared with the mussel; it is like a narrow saddle which extends only about half way over the side of the mussel shell. The position of the glochidial shell valves in Figs. 5 and 6 will be better understood from a comparison with this figure. The specimen of Fig. 6 is viewed from a ventro-lateral aspect. The following measurements (in decimal parts of millimeter) were made from the specimen represented by Fig. 6:

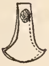
Length of glochidium	0.095 mm.
Width (height)	0.15 mm.
Length of mussel	0.320 mm.
Width (height) of mussel	0.215 mm.

The mussel, as compared with glochidium, is nearly three and one half times as long and nearly one and one half times as wide. It is evident that there must have occurred a material increase in the size of the cellular cyst of the tissue of the host which encloses the young mussel.

The shape of the mussel in the stage shown by Fig. 7 led to the suspicion that the glochidium was of *capax* instead of *lævissimus*, but the form of the glochidium is that of *lævissimus*, and there is no sign of teeth on the shell.

It is not known that such a growth in size and alteration of form occurs in any other species during the period of parasitism. What further change in form or growth in size would occur before the liberation of the mussel cannot be determined without additional material, not now available.

Biological Station,
Fairport, Iowa,
December 23, 1910.



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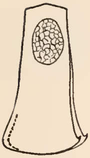

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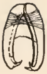

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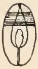

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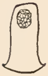

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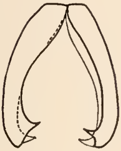

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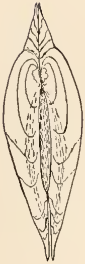

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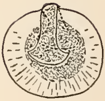

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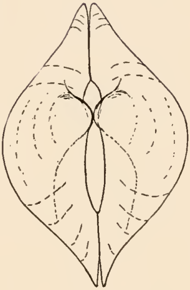

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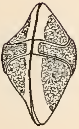

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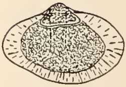

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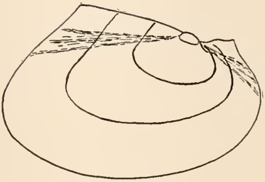

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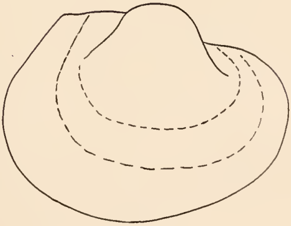

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ROBERT E. COKER AND THADDEUS SURBER

NOTES ON SOME ARACHNIDS FROM OHIO VALLEY CAVES.[1]

NORMAN E. McINDOO.

In September, 1909, immediately after the expiration of my year at the Indiana University's Cave Farm,[2] I spent two weeks collecting arachnids in the following caves: Marengo, Spring, Wyandotte, Little Wyandotte, Sibert's Well, Saltpeter and Mammoth Cave. In all these caves 268 individuals were caught. The expenses in part were defrayed by a grant from the American Association for the Advancement of Science.

Linyphia Weyeri Emerton[3] was taken only from Marengo Cave. This species in this cave is as abundant as *Troglohyphantes* (*Willibaldia*) *cavernicola* Keys. is in the Shawnee Cave. They are most abundant at a place 200 feet from the old entrance, or 500 from the new one. None were seen farther that 1,200 feet from the old entrance. Insects from this locality to the end of the cave are very rare, while near the entrance they are very abundant. The habitat, habits, webs and cocoons of this spider are similar to those of *Troglohyphantes*. Some were collected on their snares in the angles formed by the floor and wall, some along clay banks, some in little pits in the sand floor, and others under rotten boards and débris with thysanurans, beetles, diptera and myriopods. They were not sensitive to my carbide light, but were easily irritated by blowing on the web. The snares were quite abundant and were constructed like those of *Troglohyphantes*. Several cocoons were collected; one contained newly hatched spiderlings which were white in color. One spider was caught eating a thysanuran. Since the entrance to this cave is an artificial one securely made in a sink hole, we may expect an even temperature throughout the cave. And since the spiders are most abundant near the entrance we may attribute this phenomenon to the great number of insects.

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[1] Contribution No. 117 from the Zoölogical Laboratory of Indiana University.

[2] "Biology of the Shawnee Cave Spiders," BIOL. BULL., Vol. XIX., No. 6, November, 1910.

[3] These various species were identified by Dr. Alexander Petrunkevitch.

Phanetta subterranea Emerton.—A few were collected 300 feet from the entrance of Spring Cave (a wet cave one fourth mile west of Marengo Cave). These were found under flat rocks and rotten boards on the floor in damp places. They were in company with thysanurans. Several were caught in all parts of Little Wyandotte; however, none nearer the entrance than 75 feet. A few of these were observed under flat stones, but most of them were seen on the base, or in the cracks of stalagmites. Those on the base were usually running about, while those in the cracks were hanging to the underside of their tiny webs.[1] The snare is a flat sheet. It generally droops a little in the center. The meshes are very minute and the number of attachment threads depends on the surroundings. This species is a swift runner and the young are entirely white. Small diptera, thysanurans and myriopods were rather plentiful. This arachnid was abundant in Saltpeter Cave under flat rocks in moist places. They could not be affected by carbide light. Diptera and thysanurans were common.

Anthrobia mammouthia Tellkampf was found only in Mammoth Cave. Eleven specimens were caught. Since I had to keep in company with a crowd of cave visitors who were led by a guide, I was unable to stop more than one or two minutes at a place. However, I found specimens at various localities and undoubtedly they may be taken at any place in the cave where there is sufficient moisture. They were always found under flat rocks, on old mouldy paper and among débris. One small white, disk-like cocoon was observed under a flat stone. These spiders are rather active, not fast runners, and are not difficult to catch for they cling tightly to the rocks and to one's fingers. They vary in color from white to a light brown.[2] In most places insect life seemed to be comparatively scarce. Perhaps these arachnids eat old mouldy paper, remains of lunch and the limited number of small insects.

Meta menardi Latreille.—They are rather common at the mouth of Spring Cave. One was observed in Saltpeter Cave and five were caught at the end of the main channel in Mammoth

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[1] Blatchley says these are wandering spiders and spin no webs. "Indiana Caves and their Fauna," Rep. Ind. Geol. Surv., XXI., p. 204.

[2] Hitherto they have always been described as being only white.

Cave. According to the guide this locality is two and one half miles from the entrance. Concluding from the number of webs at this place, *Meta* must be common. Since *Meta* is an outside form and is ordinarily never found very far from the entrance of a cave, there is evidently an opening somewhere near this place. Here as elsewhere in this cave, cave crickets, small diptera and blind beetles were often seen.

Scotolemon flavescens Cope.—This cave harvestman was very abundant in Wyandotte. They were found some distance from the entrance on the damp floor where visitors walk, and often at the base of damp stalagmites. They were always found in the same places as thysanurans and where candle drippings were common. They probably eat the candle drippings and each other, for three couples were seen fighting and several were found dead. They are the least active arachnids I have ever seen and are not affected by carbide light. The second pair of legs, exceedingly long, are used as tactile organs. Several individuals of this species were caught in Little Wyandotte.

Phalangodes armata Tellkampf.—Two specimens of this cave harvestman were taken in Mammoth Cave, one at River Styx and the other at end of main cave. It is much larger and more active than the preceding species. It is usually found on the walls and not on the floor.

Chtonius Packardii Hagen.—One specimen of this small semiblind pseudoscorpion was taken in Wyandotte, two in Little Wyandotte and one in Mammoth. I have also taken it in Shawnee Cave. It is usually found under damp rocks. It moves along slowly with its chelæ held in the air in front, and is very difficult to find.

In order to keep the specimens from any of these caves alive very long, it was necessary to place them in a saturated atmosphere. They were most conveniently kept in small vials. One individual with a drop of water was placed in each vial. In such confinement several died in a few days, but the majority survived for a month or more. Light experiments like those with *Troglohyphantes* were prosecuted with *Theridium porteri* Banks and *Erigone infernalis* Keys., collected in Mayfield's Cave. They were decidedly negatively phototropic. Various outside forms were likewise experimented with. Some of these were negatively, others positively phototropic. The kind of phototropism depends on the natural environments of the specimen. It was observed that the specimens collected on this trip always preferred the dark end of the vials. All true cave spiders are more or less negatively phototropic. The distribution and number of all true cave spiders are controlled by even temperature and the abundance of food. They probably have no enemies other than themselves and are rarely seen fighting. They soon die outside the caves unless kept in a saturated atmosphere.

A FURTHER NOTE ON KERATOSUM COMPLEXUM.

CHARLES W. HARGITT.

In my original description of this anomalous hydroid,[1] it was stated that not only was it necessary to create for it a new genus as well as species, but that there "might be the necessity of establishing for it a new family." It was further said: "Concerning the family relations I am not disposed in this connection to enter into any critical review. While the Perisiphonidæ would be the only one under which it might be placed, still this family as at present defined . . . would by no means provide for the species. For example, while there is an axial tubular mass, as shown in Fig. 9, there is no single one of these which bears the hydrothecæ as called for by the definition referred to. However, the species may be left under this family till such time as adequate revision may be undertaken, when needed modifications may be provided."

I have since found access to a paper by W. Baldwin Spencer, entitled "A New Family of Hydroidea, Together with a Description of the Structure of a New Species of Plumularia," published in *The Transactions of the Royal Society of Victoria*, 1890, a publication of very limited circulation. Had this account been available to me at the time of my perplexity concerning the family relations of *Keratosum* it would have greatly facilitated my insight into many features which were extremely puzzling. I take the first opportunity to add to my earlier account what seems to be a solution of the point left in abeyance pending further knowledge.

Spencer established the family Hydroceratinidæ for a rather remarkable hydroid obtained from Port Phillip having much in common with *Keratosum* in both structure and habit, though with very sharp differences, the details of which need not be given here since only the matter of its family features are in question. One interesting point of coincidence may be cited, namely, that Baldwin at first referred his specimen to the family

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[1] Biol. Bull., Vol. XVII., p. 379.

Ceratelladæ, Gray, thought to belong to the sponges, but later recognized as hydroids. Further comparison convinced the author that his species could not be included under the Ceratelladæ, hence his institution of the Hydroceratinidæ. The following is his definition of the family:

"Family HYDROCERATINIDÆ."

"Hydrophyton consisting of a mass of entwined hydrorhiza, with a skeleton in the form of anastomosing chitinous tubes: the surface is studded with tubular hydrothecæ, into which the hydranths can be completely retracted. Hydranths sessile and connected with more than one hydrorhizal tube, claviform with a single verticil of filiform tentacles. Defensive zoöids present with a solid entodermal axis and nematocysts borne at the distal end."

To one who has any considerable knowledge of hydroid morphology it will hardly be necessary to point out the more obvious features in this definition which directly or approximately embody corresponding features as described in the account of *Keratosum*. There are, however, certain points in which I am not certain that the definition of Hydroceratinidæ would wholly apply to *Keratosum*. For example, it is stated that "hydranths [are] connected with more than one hydrorhizal tube." I have directed attention to the complex anastomoses of the siphonal (this term seems more accurate than Spencer's hydrorhizal) tubes but I have not been able to confirm the condition diagrammatically portrayed by his Figs. 3 and 13. It must be noted, however, that my material was not in that vital condition rendering easy and certain the demonstration of a point like the one in question. Still the tubular and hydrothecal relations were such as to render it perfectly sure that the conditions figured by Spencer are lacking in *Keratosum*.

But with this difference granted it does not seem sufficiently great to vitiate the many points of agreement which are more fundamental and characteristic as family features. There are also apparent differences as to the nematophores of the two species, yet these are rather specific than even generic and may be disregarded in this connection. Furthermore, there is not in

Keratosum any tendency toward a bilaterality in the growth habit such as described for *Clathrozoön wilsoni*. But here again we have a feature which need hardly be considered incompatible in the family aspects of the species. One finds such differences of aspect in genera and species of Gorgonidæ, to which *Clathrozoön* bears some superficial resemblance. I am constrained to believe that in its family relations *Keratosum* is more closely akin to Hydroceratinidæ than to any existing family of *Hydrozoa*, and am quite prepared to propose that it be so designated, at least until stronger reasons are found for a different disposition.

In closing, attention may be called to a still further coincidence between *Keratosum* and *Clathrozoön*, namely, the absence in both of any trace of reproductive organs. I had already emphasized this in the original description of *Keratosum*, stating that while an absence of germ cells might call for no surprise, yet "if gonangia are an organic part of the skeleton one might expect some trace of them, . . . but none could be recognized."

This further inquisition into the morphology of these hydroids tends to confirm and emphasize what had been said in concluding my original description, that "we have in this hydroid one of the most interesting, and in some ways anomalous, of this remarkable group of organisms." The foregoing comparison of the species with that from Australia, *Clathrozoön wilsoni*, tends to further accentuate this impression.

Naples Zoölogical Station,
January 3, 1911.

Vol. XX. *March, 1911.* *No. 4*

BIOLOGICAL BULLETIN

THE NESTS AND LARVÆ OF NECTURUS.

BERTRAM G. SMITH.

THE NESTS.

1. *Nests in a Lake Habitat.*—Through the courtesy of Professor Bennet M. Allen I recently became acquainted with the spawning grounds of *Necturus maculosus* Rafinesque in Lake Monona, Wis. During the latter part of June and the early part of July, 1910, several trips were made to the locality for the purpose of obtaining embryological material.

The "nests" were found in water about 3–5 feet deep and about 50–100 feet from the shore, in a locality where the bottom was strewn with loose flat stones of various sizes. The largest of these stones, about 1½–2 feet in diameter, frequently served as cover for the eggs of *Necturus*. The eggs are attached by the slender stalks of the gelatinous envelopes singly to the under sides of these stones, distributed over an area about 8–10 inches in diameter (see Fig. 1). The presence of minute algæ, etc., in the water made it so opaque that it was impossible to see the bottom; the eggs were obtained by wading in the water, feeling about with the feet for a large flat stone, then bringing it to the surface.

Eycleshymer ('06) describes nests of eggs attached to the under sides of logs, boards, pieces of tin, canvas, etc., but does not mention finding nests under stones. Doubtless any convenient object may be selected as cover.

The number of eggs present in a nest was determined in five cases as follows: 18, 61, 80, 84, 87. The average is 66. The nest photographed contained 84 eggs.

The first embryos were obtained on June 22; these were in an advanced stage of development, with head well formed and a small tail rudiment. The latest embryos were collected on July 5; at this time nearly all the embryos had hatched, only a few unhatched embryos being found in each of several nests. The empty capsules remained attached by their stalks.

Eycleshymer ('06) states: "The time of egg-laying varies in different lakes, depending upon the time when the temperature of the water reaches a certain degree. In the larger, deeper lakes with bold shores this is much later than in those possessing wide shoals. . . . According to Professor Whitman's and my own experience the best time for collecting is during the middle and latter parts of the month of May. The writer has collected eggs as early as May 3, and as late as June 5, but these extremes mark the beginning and closing of the early and late seasons." Unless the dates given are for *newly-laid* eggs, which would

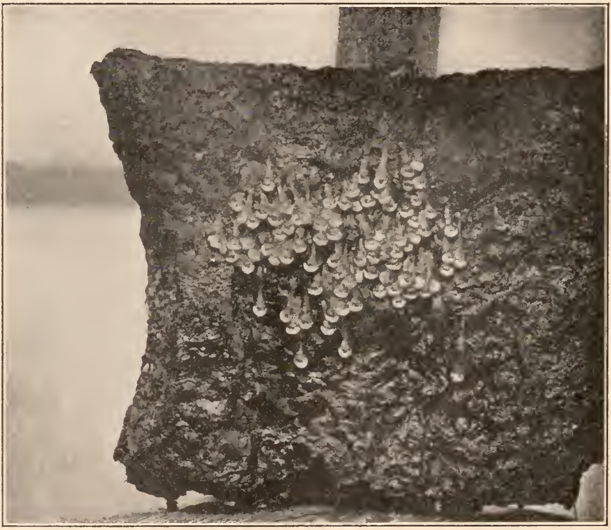

FIG. 1. "Nest" of eggs of *Necturus*. The stone to which the eggs are attached has been removed from the water and set on edge on the wharf; it is about 16 inches in diameter. The embryos are in an advanced stage of development.

hardly be inferred from the text, my experience shows that the collecting season may be much later than Eycleshymer recorded.

A noticeable feature of the development as compared with other amphibians that I have studied, is the uniformity in the stage of development of embryos found in different nests in the same locality. On each of the following dates from four to seven nests were secured: June 22, 25, 29, July 5. On each date all the eggs were found so nearly in the same stage of development that only slight differences could be detected in eggs from different nests. This uniformity points to a very short spawning season—perhaps two or three days—in this locality; it would seem that all the eggs in a restricted area are laid at nearly the same time. But Eycleshymer ('06) says: "The eggs are first deposited in those localities where the water is shallow and exposed for the greater part of the day to the rays of the sun. The period of egg-laying usually covers two or three weeks. There is no foundation whatever for the statement made by Hans Virchow that the animals deposit their eggs so to speak at the same hour."

I had no success in keeping embryos of *Necturus* alive in the laboratory, although later in the year larvæ of *Cryptobranchus* thrived under the same conditions.

2. *Nests in a Stream Habitat.*—I am not aware of any published observations on the nesting of *Necturus* in streams, hence the following notes on the subject may be of interest.

During the late summer and early autumn of the past five years, while searching for adults, eggs and larvæ of *Cryptobranchus*, I have had occasion to overturn numberless stones in the bottom of a large creek tributary to the Allegheny River, in northwestern Pennsylvania. This has resulted in the frequent finding of specimens of *Necturus*.

All the specimens found were small, none exceeding 20 cm. (8 in.) in length and most of them were much smaller. The smallest, taken September 13, 1906, was 35 mm. long (see Fig. 6). This specimen was one of a group of six or seven found under the same stone; the others, aided by the swift current, escaped.

These circumstances led me to suspect that the stream was a spawning ground for *Necturus*. This suspicion received confirmation when, on August 24, 1910, I found attached to the under side of a large rock about fifteen or twenty empty egg capsules of *Necturus*, all in good condition. At the distal end of each capsule was a large round hole through which the embryo had escaped.

THE LARVÆ.

1. *The Embryo at the Time of Hatching.*—Fig. 2 is from a photograph of an embryo of *Necturus* obtained from Lake Monona on July 5, 1910. This embryo was apparently ready to hatch, since nearly all the other embryos in the nest had already hatched out.

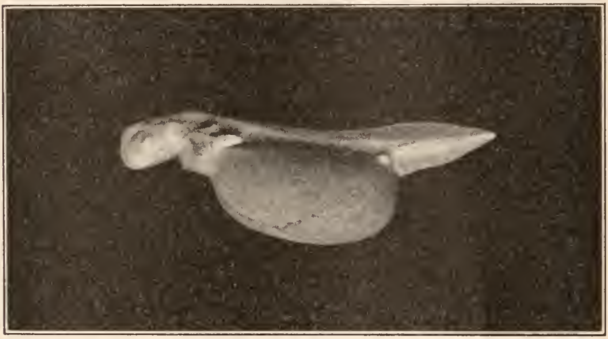

FIG. 2. *Necturus* embryo ready to hatch, killed in Tellyesnicky's fluid and preserved in formalin, July 5, 1910. (× 3½.) From Lake Monona.

The embryos of *Necturus* are hatched in a less advanced stage of development than is the case with *Cryptobranchus* (see Smith, '07, Fig. 9); but in *Cryptobranchus* at least there is considerable variation in the time of hatching and the figure referred to represents one of the more advanced of the newly-hatched embryos, hence the difference may be less than the figures indicate. *Necturus* is hatched in water of a much higher temperature than is the case with *Cryptobranchus*, and this would naturally tend to soften the gelatinous envelope and aid in the early escape of the embryo.

In *Necturus* the large yolk content at the time of hatching is even more noteworthy than in *Cryptobranchus*. Though set free as a larva, the animal is really in an embryonic state so far as its food supply is concerned, until long after its liberation. The only advantages secured by hatching in this condition would seem to be better aeration and opportunities for exercise; these advantages must be in part offset by the increased danger of capture by some larger animal.

Aside from differences in the stage of development, the embryos of *Necturus* and *Cryptobranchus* at about the time of hatching are so much alike that it would be difficult to tell them apart were it not for the noticeably greater bulk of the latter. At the time of hatching the *Necturus* embryo measures about 18 mm. in length, the *Cryptobranchus* embryo about 24 mm.; this difference may be due in part to the more advanced condition of the latter, but throughout the entire embryonic history the size of embryos of corresponding stages is so much greater in the case of *Cryptobranchus* that though eggs from different nests of the same species may vary slightly in size, the extremes of variation of the two species do not overlap.

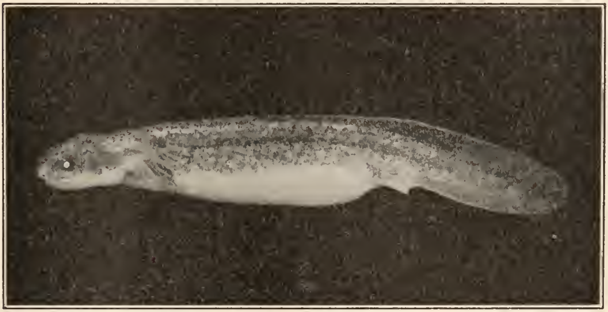

FIG. 3. *Necturus* larva reared from advanced embryo obtained from Lake Monona, and preserved in formalin July 31, 1910. (× 3½.)

2. *Larval Development.*—I have examined a series of early larval stages kindly loaned me for the purpose by Dr. Bennet M. Allen. The series comprises twelve specimens raised from advanced embryos obtained from Lake Monona and preserved in formalin on the following dates: July 31, August 19, 21, 25, 1910.

Specimens preserved July 31 (see Fig. 3) average about 25 mm. long. As compared with *Cryptobranchus* larvæ of the same age after hatching, in *Necturus* the general form of the body is more slender, except that the yolk sac is of relatively greater size. The absolute size of the *Cryptobranchus* larva is much greater. The pigmentation in *Necturus* is less intense, and the general appearance is that of a less advanced stage of development.

These specimens show the beginning of the dorso-lateral stripes, which attain great prominence in later stages and will be described in the stage in which they are most marked.

In the larvæ preserved during the latter part of August, the yolk sac still persists, though its size is so reduced that the abdomen is only slightly distended by it. The lateral stripes are quite distinct, though not so conspicuous as in slightly later stages. The larvæ average about 30 mm. in length. In *Cryptobranchus* larvæ of the same age after hatching, the yolk sac is so reduced that the abdomen is no more distended than in the adult; the color is much darker than in *Necturus*, the form of the body stouter, and the absolute size nearly twice as great.

For a larval specimen 34 mm. long, I am indebted to Mr. L. W. Harrington, formerly an assistant in the Zoölogical Laboratory of the University of Michigan. This specimen was obtained by Mr. Harrington from fishermen in the Detroit River on November 24, 1906, and was examined by me on the same day, before preservation. The transparency of the ventral abdominal wall enables one readily to note that the yolk has been entirely absorbed. Since the coloration conforms accurately to that of the Lake Monona specimens, a description of the color pattern will serve for all the western larvæ examined by me.

As contrasted with the adult, the most striking feature of the 34-mm. larva is the presence of a conspicuous dorso-lateral longitudinal stripe, light yellow in color, on each side of the body. The lateral margin of each stripe is metamerically crenate. The body stripes continue unbroken and without fusion to the tip of the tail; anteriorly, they are separated by a slight break from similar stripes along the margin of the dorsal surface of the head; these latter are sometimes connected at their anterior ends by a faint transverse bar over the tip of the snout.

The ground color of the dorsal and lateral surfaces is a dark brown, with small scattering spots of lighter color especially noticeable along the sides of the body and tail. As compared with the earlier stages the pigmentation is much more intense, but this merely serves to accentuate the light-yellow stripes. It is to be particularly noted that in all the western larvæ that I have examined, the dark color of the dorsal surface of the body is continued along the dorsal edge of the tail between the lateral stripes (see Fig. 4). The ventral surface is pale yellow, almost transparent.

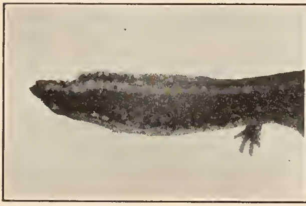

FIG. 4.

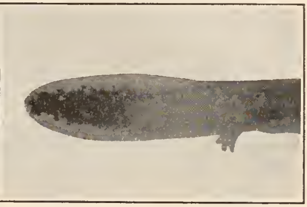

FIG. 5.

FIG. 4. Caudal portion of a 34-mm. *Necturus* larva taken from the Detroit River, showing color pattern of the tail. The specimen is somewhat shrivelled from partial drying before preservation; in particular the ventral margin of the tail is upturned. Photographed after preservation in formalin. (× 3½.)

FIG. 5. Caudal portion of a 35-mm. larva of *Necturus* taken from a stream in northwestern Pennsylvania, showing color pattern of the tail. Photographed from a formalin specimen. (× 3½.)

Mention has already been made of larvæ taken from a stream habitat in northwestern Pennsylvania. No attempt was made to capture all the specimens found, but a series was preserved representing a gradation in size from the smallest, 35 mm. long, to the largest, 20 cm. in length. Of these the fourteen smallest, all under 15 cm. in length, show larval characteristics in the color pattern.

The smallest specimen, 35 mm. in length (see Fig. 6), was taken on September 13, 1906. This larva is younger and morphologically less advanced than the slightly smaller larva obtained from the Detroit River; but on account of an important difference in the color pattern its description has been deferred.

By dissection it was found that the yolk sac, though reduced almost to the form of a digestive tube, still contained a small amount of yolk. Assuming that this larva had been hatched about 8–10 weeks, we find a similar condition of the yolk sac in *Cryptobranchus* larvæ of the same age after hatching.

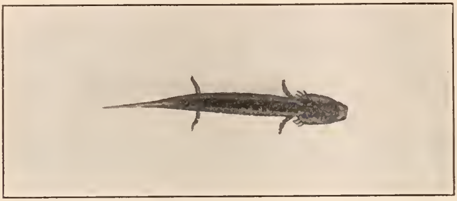

FIG. 6. Living larva of *Necturus*, 35 mm. long. (× $\frac{7}{8}$.)

This specimen differs from the western larvæ in that the dorsolateral stripes unite in the median line at the base of the tail, to be continued as a single stripe along the dorsal edge of the tail (see Fig. 5). Since this peculiarity is present in all the fourteen larvæ that I have examined from the eastern habitat, it would appear to be a constant difference between the eastern and western forms. Otherwise the color and color pattern of both eastern and western larvæ are the same.

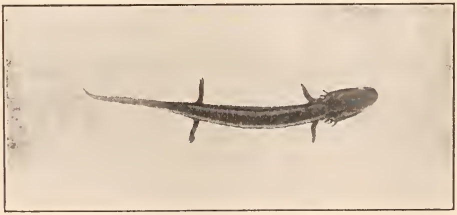

FIG. 7. Larva of *Necturus*, 55 mm. long, photographed after preservation in ormalin. Nearly actual size.

The larger specimens may be described as follows: A 42-mm. larva taken on August 29, 1907, differs morphologically from the 35-mm. larva in its slightly greater yolk content; evidently it is younger, though of greater size. A specimen 55 mm. long

(Fig. 7), taken on August 20, 1906, agrees in its color and color pattern with the 35-mm. larva; the yolk has been entirely absorbed. Another 55-mm. larva taken September 13, 1906, is in the same condition. A 60-mm. specimen taken August 19, 1910, shows a slight loss in the distinctness of the stripes.

The striped pattern characteristic of the larvæ reaches its culmination in specimens of about 55 mm. body length. From this time on it gradually disappears, the light yellow stripes being obscured by dark pigment; during the same period the large black spots, which give the mottled appearance to the color pattern of the adult, become established. With the attainment of a body length of 15 cm. the larval color pattern is entirely replaced by that of the adult. Several specimens with a body length of 20 cm. were dissected and found to be sexually mature.

Eycleshymer ('06), after describing the variation in color of the adult, continues: "It is probable that these variations in color are responsible for a number of specific names. As an instance I might state that some years ago Dr. Garnier ('88) described a small *Necturus*, taken from the Maitland and Lucknow Rivers in Ontario, to which he gave the name *Menobranchus lateralis*, var. *latastei*. 'The coloration above was black, the abdomen sooty and the gular fold white.' During the summer of 1904 the writer was fortunate enough to secure two young animals which measured about 4 and 6 inches respectively. The smaller corresponds closely to the description given by Dr. Garnier and there is every reason for believing that the animal in question is the young of *Necturus maculosus*. The older of the two presents the general coloration of the adult. That *Necturus* should undergo such striking changes in color may appear remarkable to one who has not studied the early stages but when one has followed the changes in color pattern during growth he finds that they are no less striking and remarkable than in the birds."

While I do not doubt the evidence of great variability in the color and color pattern during growth, furnished by those[1] who have reared *Necturus* from the eggs, the fact remains that in all the specimens studied by me not a single non-striped larva has been found, nor is any specimen really black. Moreover, I would point out that Dr. Garnier's description above quoted would make an excellent account of the coloration of a year-old larva of *Cryptobranchus alleghenieniensis*, having external gills and with a body length of 8 cm.

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[1] In a letter from Dr. Whitman to the writer, dated April 22, 1907, he says: "I have reared *Necturus* from the egg, and I can assure you that now and then a single dark *brown* individual is found among the striped ones. I raised one from the egg, and have had two captured by net. They are rare, but are unquestionably *Necturus* in the cases mentioned."

Zoölogical Laboratory,
University of Wisconsin.

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A 74 MM. POLYODON.

C. H. DANFORTH.

(From the Anatomical Laboratory of Washington University.)

The developmental stages of *Polyodon* have long been sought by biologists but, so far as published accounts show, neither the fertilized eggs nor the young embryos have ever been seen. For this reason it seems advisable to announce the capture of a specimen smaller than any previously recorded.

The individual in question was taken from the Mississippi River near St. Louis on July 12, 1910. It was immediately killed and fixed in Zenker's fluid and hardened in alcohol. In the latter medium it measured 74 mm. from the tip of its snout to the tip of its tail. The figures show three views of this embryo after preservation.

The features in which it seems to differ most from the adult are the relatively large barbels, the rostrum, and the fins and tail. The general outline of the body, too, appears rather more fusiform, but a number of measurements failed to bring out any marked peculiarities in this respect.

The barbels, represented in Figs. 1 and 2, are approximately a millimeter long, or .013 of the total length of the fish. This is relatively many times their adult size. They are, however, small and apparently rudimentary even in the young, and a macroscopic examination of them revealed no new points of interest. In fish of 170–175 mm. they are still relatively large as compared with the adult.

The rostrum of the embryo is somewhat unlike that of the adult in outline. In the former (cf. Figs. 2 and 3) it tapers in width from the nostril to the tip with only a slight constriction near the middle, whereas in the adult there is a characteristic and generally well marked constriction of the proximal half and usually a distinct dilation of the distal half, giving the whole its typical paddle-shaped appearance. In an embryo of 89 mm. the rostrum is similar to that of the 74-mm. specimen, but in individuals of 170 mm. and over the organ is much more truly spatulate in outline. With the exception of the specimen under consideration, the width of the rostrum was found to be relatively greater in the small fish than in the larger ones, but width of snout and contour are not very closely correlated so that some rather wide snouts are not very spatulate in outline.

The length of the rostrum at different ages is shown in the accompanying table based on measurements of twenty specimens, ten varying in length from 74 mm. to 200 mm., and ten from 1,000 mm. to 1,300 mm. Intermediate stages were not available. In this table the second column gives the total length from tip of snout to tip of tail, the third gives the length of the rostrum measured from its tip to a line passing between the anterior corners of the eyes, and the fifth column shows the width of the rostrum at the beginning of its distal third. The decimals in the sixth column are obtained in each case by dividing the length of the rostrum by the total length of the fish. Professor Stockard[1] measured in this way a number of adult *Polyodon* from 24 in. (about 600 mm.) to 69 in. (about 1,725 mm.) and found an interesting correlation between the size of the fish and the length

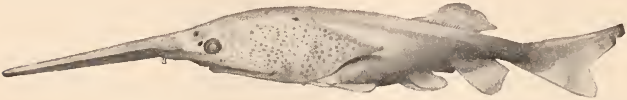

FIG. 1. Lateral view of a specimen of *Polyodon spathula* measuring 74 mm. total length.

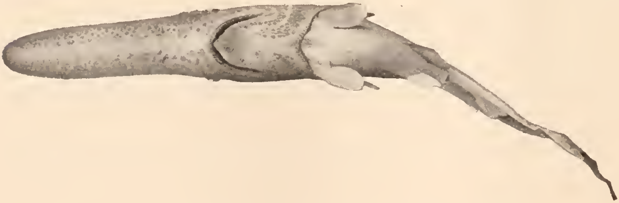

FIG. 2. Ventral view of the specimen shown in Fig. 1.

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[1] Stockard, Charles R., "Observations on the Natural History of *Polyodon spathula*," *Amer. Nat.*, Vol. XLI., pp. 753–766, 1907.

of the rostrum. His tables show a steady increase in the relative length of the rostrum in passing from larger to smaller individuals. My own measurements reveal the presence of somewhat striking individual variations, but it will be seen, nevertheless, on examining the table that in the first ten the length of the snout is, on the whole, gradually increasing while with the latter ten the reverse is the case. The smallest fish measured by Professor Stockard was approximately 600 mm. in length and had a rostrum represented by the fraction .333. The largest of the small fish measured for the table presented herewith was 200 mm. long with a rostrum represented by .390. The available data, therefore, seem to indicate that the rostrum attains its maximum development at a time when the fish is from 200 mm. to 600 mm. in length. The biology of the species is too little known to enable one to say whether or not there is any functional significance in such a development at this time.

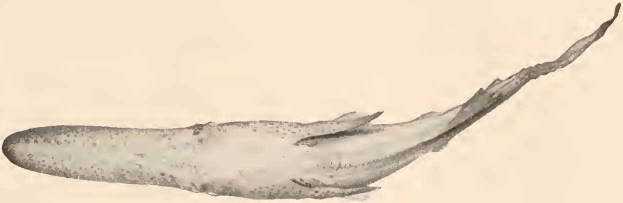

FIG. 3. Dorsal view of the same fish.

In adult *Polyodon* the fins show a marked angularity. In the embryo they are rounded and much less distinctive. This difference will become evident on comparing the figures accompanying Professor Stockard's article with those presented here. The position of the various fins does not seem to differ greatly between the small and the large fish.

The tail of the embryo is rather strikingly unlike that of the adult. It is much more obviously heterocercal and the dorsal part is somewhat lobed as shown indistinctly in Fig. 1. The small terminal lobe, which is not found in the adult, seems to be either without rays or with rays only feebly developed. It is a fleshy mass with apparently no tendency to become elongated into a slender filament. It is not clear from the material at

TABLE OF MEASUREMENTS.

Fixing Fluid.	Total Length, mm.	Length of Rostrum, mm.	Length of Operculum, mm.	Width of Rostrum, mm.	Rostrum by Total Length, Decimal.
Zenker's	74	22	19	6	.297
Formalin	89	26	21	9	.292
Zenker's	91	27	22	8	.297
Formalin	104	34	27	11	.327
"	107	34	28	12	.318
"	140	48	35	16	.343
"	144	49	36	16	.340
"	170	58	46	18	.341
"	175	58	46	18	.331
"	200	78	50	20	.390
"	1,000	260	300	72	.260
"	1,030	290	280	80	.282
"	1,050	310	300	75	.295
"	1,070	260	290	72	.243
"	1,090	300	300	78	.275
"	1,180	320	340	80	.271
"	1,200	340	360	80	.283
"	1,210	330	370	90	.273
"	1,210	310	330	80	.256
"	1,300	330	370	75	.254

hand whether this portion disappears with growth or becomes incorporated with the rest.

Measurements were made upon several other parts of the body but the data obtained do not seem to indicate any special peculiarities beyond those already mentioned. Of these measurements the length of the operculum is alone included in the table, the figures in the fourth column representing the distance in millimeters from the posterior corner of the eye to the tip of the opercular flap.

The age of the specimen cannot, of course, be stated, but it seems probable that it is from the brood of the current season. Professor Stockard's observations indicate an early spawning. During the summer some specimens only slightly larger than the one described were taken, while others had reached 175 mm. or more in length, and in the winter specimens from six to ten inches long are captured. Consequently, if these fragmentary observations are to be trusted, it seems probable that these fish hatch early in the spring and grow rapidly during the first year, attaining a length of possibly ten inches in this time.

The writer is indebted to Drs. R. J. Terry and V. E. Emmel for securing and preserving the specimens studied. The drawings are the work of Mr. W. T. Oliver.

STUDIES ON SEX-DETERMINATION IN AMPHIBIANS.

IV. The Effects of External Factors, Acting Before or During the Time of Fertilization, on the Sex Ratio of *Bufo lentiginosus*.

HELEN DEAN KING,

The Wistar Institute.

At the present time practically all of the investigators who are working on the problem of sex-determination believe that the sex of a zygote is fixed at a very early period, and that it is not dependent upon environmental factors acting during embryonic development. There is considerable diversity of opinion, however, regarding the time when sexual differentiation takes place and the way in which it is brought about.

A number of investigators, among whom may be mentioned Rauber ('00), Beard ('02), Schultze ('03) and Russo ('09) maintain that sex is determined in the ovary, chiefly by nutritive conditions. According to this view mature eggs are either male or female, and the spermatozoan can take no part whatever in determining the sex of the egg it fertilizes.

Other investigators, prominent among whom are Stevens ('05), Wilson ('06) and Morgan ('07, '09, '10), are inclined to the opinion that sex is not determined until the time of fertilization, and that it is the spermatozoan that definitely and unalterably fixes the sex of the individual.

These two views are apparently irreconcilable, and such an array of seemingly indisputable facts has been brought forward in support of each of them that it may be, as Jordan ('09) has recently suggested, that "sex has been attained by several paths, and is now determined in different modes and at different times in the different groups of animals and plants." If this be true, it will be futile to try to find a single factor, or set of factors, that is the fundamental cause of sex in all organisms; and investigations in this field must be confined to an attempt to discover the conditions that determine sex in the particular form selected for study.

The important experiments of Tower ('10) with the eggs of the potato-beetle, *Leptinotarsa*, and also those of Tennent ('10) with cross-fertilized echinoderm eggs, have shown that very definite alterations in the structure of the developing organism can be produced by changing the external conditions that surround the egg at the time of fertilization. The recent investigations of Shull ('10*a*, '10*b*) and those of Whitney ('10) on the rotifer, *Hydatina senta*, indicate that the chemical composition of the water in which the cultures live determines the appearance of the sexual cycle. But what the substances are that bring about the appearance of the sexual forms, and how they act, has not yet been ascertained.

All of these experiments show that environmental factors can greatly change the course of development even if they act for only a comparatively short period on the unsegmented egg: whether they can influence anything as fundamental as sex, however, remains to be determined. It was for the purpose of testing this point that a series of experiments on the eggs of the toad, *Bufo lentiginosus*, was undertaken two years ago. The first of these experiments showed pretty conclusively that temperature, acting at the time of fertilization is not the dominant factor in the determination of sex in this form (King, '10). The present paper records the results of the second series of experiments along this line. It is hoped that the method of investigation employed will eventually settle the point as to whether sex in *Bufo* is determined at or before the time of fertilization, even if it gives no clue to the factors that are involved in this process.

The Wistar Institute of Anatomy and Biology has provided an equipment for the carrying on of these investigations that is nearly ideal for the purpose. This equipment consists of two iron racks, each of which supports three tiers of twenty galvanized iron trays 9×12×2½ inches: dishes of this size are convenient for handling and each gives ample space for fifty large tadpoles. A series of ¾-inch iron pipes, connected with the city water system, are attached to the supporting frame five inches above each tier of trays; and by means of small stop-cocks each dish is supplied with running water. The depth of the water in the trays is kept at about two inches by means of constant level siphons, the water running from each siphon into a trough that empties into a large exit pipe. By the use of this apparatus large numbers of eggs, subjected to very different influences at the time of fertilization, can be reared under similar external conditions. Marked variations in the results of the several series of experiments must be attributed to the different conditions under which the eggs were fertilized; they cannot be due to differences in the environmental conditions under which the embryos developed.

The apparatus described above was used for all of the experiments made during the spring of 1910. An abundant supply of varous kinds of water plants was always kept in the dishes containing the tadpoles. All of the tadpoles received similar food, which consisted of small pieces of cooked meat or of frog muscle, yolk of egg, cereal and so-called "fish food." The water was allowed to run slowly in each dish for about six hours every day, and was then turned entirely off at night to prevent a possible accident through a change in the water pressure. To prevent any loss of tadpoles through a fouling of the water by food and impurities that could not be carried away through the siphon, the inner end of which was covered with fine netting, each dish was thoroughly cleaned once a week during the first month after the experiment was started, and much oftener when the tadpoles had become larger and the weather warm.

As many as possible of the individuals used in each series of experiments were carried through to metamorphosis and their sex ascertained: the methods used in ascertaining sex being the same as those previously employed (King, '07).

In the various tables given in this paper the figures in the second column refer in every case to the number of individuals alive one week after the experiment began; they do not indicate the number of eggs that were subjected to the unusual conditions at the time of fertilization. In investigations of this kind it is very important, as I am fully aware, that the mortality in the various series should be known and taken into account in considering the results. With the large numbers of eggs that were necessarily used in these studies it was quite impossible to take the time to make an accurate count of the eggs when the experiments were made. For the eggs of the toad, when taken from the uterus, are so closely packed together in long strings of thick jelly-like substance that they are greatly distorted in shape, and a rather careful examination with a lens would be necessary to enable one to tell the exact number of eggs in any given lot.

It is possible that distinct variations in the sex ratios might be obtained when batches of eggs from different females are fertilized with sperm from different males. A control for each series of experiments was therefore considered necessary, and was obtained by fertilizing eggs from the same female with sperm from the same male in ordinary tap water. The percentage of females in this control lot was taken as the standard by which to judge whether the sex ratio had been altered by the changed environmental conditions to which the particular lot of eggs had been subjected at the time of fertilization.

1. The Influence of Alcohol on the Sex Ratio of *Bufo*.

To subject eggs to the action of solutions that would produce marked alterations in the cell structure would seem to be an easy way of ascertaining whether sex can be influenced by external conditions acting at the time of fertilization. Unfortunately the unfertilized eggs of amphibians, as well as those of the different invertebrates that are extensively used for experimental purposes, are very sensitive to the action of various substances, even when used in very dilute solutions for only short periods of time. It is therefore not possible to put the eggs of *Bufo* in any solution strong enough to greatly modify the cell structure without causing immediate death or inducing changes that prevent normal development.

Experiments made by Morgan and Tsuda ('93) show that the eggs of the frog, and also those of the toad, can develop normally for some time if they are placed in a 2.5 per cent. solution of alcohol, although they are not able to live in a 5 per cent. solution. As eggs and sperm, according to Overton ('02), are equally permeable to the lower alcohols and are not greatly injured by them, the idea was suggested that it might be possible to fertilize the eggs of *Bufo* in solutions of alcohol that would be strong enough to enter the eggs and, by combining with the fats, bring about an alteration in the cell structure that would later manifest itself in a change in the normal sex ratio. Batches of eggs from the same female were therefore placed with sperm from the same male in alcohol solutions of the following strengths: 10, 5, 2, 1, .5, .25, .13 per cent. In each case the eggs remained in the solution for one half hour and were then transferred into fresh water where they continued their development.

None of the eggs that were subjected to the action of the 10 per cent. and the 5 per cent. alcohol segmented. This result can probably be attributed to the injurious effects of the solutions on the spermatozoa which were thus rendered incapable of fertilizing the eggs. Overton ('02) has shown that a 5 per cent. solution of alcohol anæsthetizes muscle, and presumably a solution of this strength would have the same effect on the spermatozoa of *Bufo;* no investigation of this point was made. At least one half of the eggs that were fertilized in the 2 per cent. and in the 1 per cent. solutions of alcohol segmented normally and continued their development: in the remaining series practically all the eggs were fertilized.

Table I. shows the results obtained in this series of investigations.

TABLE I.

EGGS FERTILIZED IN SOLUTIONS OF ALCOHOL.

Per Cent. Alcohol.	Total No. Individuals.	No. Sex Ascertained.	Males.	Females.	Per Cent. Females.
2	398	134	68	66	49.25
1	326	130	69	61	46.92
.5	260	177	81	96	54.23
.25	244	188	95	93	49.52
.13	311	209	100	109	52.15
Control,	210	134	65	69	51.41

The sex ratios obtained in this series are very uniform, since in no case is there a deviation of even five from the percentage of females found in the control lot. Somewhat lower percentages of females were found among the individuals that had developed from eggs that were fertilized in the stronger solutions; but the deviations of these percentages from that of the control are much too small to be considered as significant, particularly as the lowest proportion of females occurs in the lot in which the eggs has been fertilized in 1 per cent. alcohol and not in the lot where fertilization of the eggs took place in 2 per cent. alcohol. The relatively high percentage of females (54.23) that is found where the eggs had been fertilized in .5 per cent. alcohol is quite within the limits of normal variations in the sex ratio of *Bufo*. For previous studies in which the sex of nearly 10,000 young toads was ascertained (King, '07, '09, '10) show that in any large lot of individuals there is a slight excess of females, as a rule, the proportion of females varying from 51 to 56 per cent. It is evident from the sex ratios obtained in this series of experiments that alcohol, in the strengths used and for the time it acted, has no influence on the determination of sex in *Bufo*.

The growth of the tadpoles that developed from the eggs that were fertilized in 2 per cent. alcohol was greatly retarded. This was not apparent during the first month after the experiment was made, but became very noticeable later. At the time of metamorphosis the majority of these tadpoles had a body length of only 7–8 mm., although the body length of tadpoles of *Bufo* at this period is normally 11–14 mm. During early development the mortality among these individuals was comparatively slight, being not more than 10 per cent. At the approach of metamorphosis the tadpoles, especially the smaller ones, began dying in great numbers; and during the last week of June over fifty of them died from no apparent cause. In no other series of experiments was the mortality among mature tadpoles anywhere near as great as in this case. Alcohol, therefore, although acting for only a short time at the fertilization period in but a 2 per cent. solution, must have so affected many of the tadpoles that they were rendered incapable of undergoing metamorphosis. All of the tadpoles in this lot were normal as far as could be determined without a microscopic examination of the various organs. None of them showed defects in the central nervous system comparable to those that Stockard ('09, '10) has found can be induced in embryos of fundulus by subjecting the fertilized egg to the action of 3 per cent. alcohol. As many as possible of the tadpoles that died at the time of metamorphosis were preserved; and sections were made of their gonads in the hope that it would be possible to ascertain the sex of the individuals by this means. In very few cases, however, were the gonads sufficiently developed to make a definite determination of sex possible.

The stunting of the individuals and the mortality at the time of metamorphosis was much less evident where the eggs had been fertilized in 1 per cent. alcohol; while in the other lots in the series the tadpoles were as large and as vigorous as were those in the control. In lots of tadpoles that have developed from normally fertilized eggs under similar external conditions there is always considerable variation in the size of the individuals of the same age. Although, as a rule, most of the tadpoles are of medium size, it not infrequently happens that a few giant individuals may be found that are nearly twice as large as the smallest individuals in the lot. It is, therefore, only when the majority of the individuals show marked deviations from the usual size that one can safely attribute the change to the unusual conditions to which the eggs have been subjected.

2. The Influence of the Spermatozoan on the Determination of Sex in *Bufo*.

In his latest contribution to the subject of sex-determination Hertwig ('07) describes a series of experiments in which the eggs of two different frogs were divided into six lots and fertilized with sperm from six different males. The mortality in several of these lots of eggs was very great, and there was no uniformity in the sex ratios that were obtained. Some cultures gave over 90 per cent. of females; others produced an excess of males. In spite of this lack of uniformity, Hertwig considers that the results indicate that the male has some influence in determining sex in *Rana*, although he is of the opinion that, as a rule, "die Eier zur Zeit der Befruchtung sexuell in so hohem Grad determiniert sind, dass der relativ geringe Einfluss des Samens gar nicht zur Geltung kommen wurde."

Morgan ('08) has suggested that, if there be a dimorphism of the spermatozoa of amphibians that is associated with sex-determination, it is possible that more sperm of one kind are produced in some individuals than in others, and that distinct differences in the sex ratios might be obtained if lots of eggs from the same female are fertilized with sperm from different males. There is the possibility, also, that the spermatozoa of one kind, if two kinds exist, are produced only in one testicle, or perhaps in considerably greater numbers in one testicle than in the other. It seemed advisable, therefore, in investigating the possible influence of the spermatozoan on the determination of sex in *Bufo* to carry out the experiment in the following way: a batch of eggs from the right uterus of a female was divided into four lots of from 300–400 eggs each. Each lot of eggs was then fertilized with sperm from the right or from the left testicle of one of three different males.

The sex ratios obtained in this series of experiments are indicated in Table II. In this table the males from which the spermatozoa were obtained are designated as 1, 2, 3; while the letters R.T. and L.T. refer to the right and to the left testicle respectively.

TABLE II.

Eggs Fertilized with Sperm from Different Males.

Males Used.	Total No. Individuals.	No. Sex Ascertained.	Males.	Females.	Per Cent. Females.
1 (L.T.)	255	126	66	60	47.61
1 (R.T.)	403	224	108	116	51.78
2 (L.T.)	380	301	143	158	52.49
3 (R.T.)	221	124	55	69	55.64

Somewhat different percentages of females were obtained in the various lots, as might be expected in any case. As the series stands in the above table, the greatest difference between the percentages of females in any two lots is but 8.03 per cent. If the percentage of females in all of the individuals that developed from eggs that were fertilized with sperm from male 1 (50.28 per cent.) is substituted for the relatively low percentage (47.61) found in the lot where sperm from only the left testicle had been used for fertilization, the difference between the percentages of females at the two extremes of the series falls to 5.36 per cent., which is only 1.19 per cent. greater than that (4.17) found between the two lots in which the eggs had been fertilized with sperm from the right and from the left testicle of the same individual (male 1). Variations in the percentages of females as great as those shown in the above table have been obtained in other cases where batches of eggs from the same female were fertilized with sperm from the *same* male (Table I.). The results of this experiment, therefore, give no very definite support to Morgan's contention that the male is probably the sex determining factor in amphibians as it appears to be in some of the lower forms. On the other hand, the experiment proves nothing against this theory. For a comparison of the percentages of females in the lot of individuals produced from eggs that were fertilized with sperm from the left testicle of male 1 with that of the corresponding lot in which sperm from the left testicle of male 2 was used shows a difference of but 4.88 per cent.; in the two instances in which sperm from the right testicle was used to fertilize the eggs, the difference in the percentages of females among the young toads is 3.86 per cent.; where one lot of eggs was fertilized with sperm from the right testicle and another lot with sperm from the left testicle of the same individual, there is later a difference of but 4.17 per cent. in the percentages of females in the two lots. It seems evident, therefore, that both kinds of spermatozoa, if two kinds exist, must be produced in approximately equal numbers in each testicle of every male. On the theory that the male is the sex-determining factor in amphibians one would therefore expect to find practically equal proportions of the sexes in any large lot of individuals, whether the eggs had been fertilized with sperm from the same or from different males.

The view that males are produced by the fertilization of eggs with spermatozoa from the right testicle, while females result if eggs are fertilized with spermatozoa from the left testicle has recently been advocated by Von Seligson ('95*a*, '95*b*). This theory, which is generally credited to Hippocratės (460–377 B.C.), is not adequate to explain the determination of sex in *Bufo* since, as shown in the above table, males and females are produced in approximately equal numbers when lots of eggs from the same female are fertilized with sperm from the right or from the left testicle of the same individual.

Of the many investigators who maintain that sex is already determined in the ovary, only von Seligson and Dawson ('09) have advocated the view that the right ovary produces male eggs exclusively and that eggs which develop into females must be derived from the left ovary. Von Seligson believes that only the spermatozoa from the right testicle are able to fertilize male eggs, and, conversely, that eggs from the left ovary must be fertilized by spermatozoa from the left testicle. This, of course, is selective fertilization for which at present there is but little evidence. Dawson is of the opinion that the spermatozoan takes no part whatever in determining sex. All of the eggs that were used in the experiments described above were taken from the right uterus of the same female. They should, therefore, according to Dawson, have produced a great preponderance of males. On the theory advocated by von Seligson two of the lots should have produced a great excess of males; and, unless we assume a struggle for existence between the sex-determining factors in the egg and those in the sperm with a final dominance of one sex or the other, it is difficult to see how the tadpole in the remaining two lots could have any sex at all.

I have not suggested the possibility that in any of these lots the individuals would be exclusively males according to the views of von Seligson and of Dawson, since it is very probable that the right uterus of the toad, and also that of the frog, contains eggs from the left as well as from the right ovary. The eggs of these amphibians break through the walls of the ovary shortly after the time that the germinal vesicle has disappeared preparatory to the formation of the first polar spindle, and they come to lie freely in the body cavity from whence they pass through the oviducts into the uteri. It is very probable that all of the mature eggs are expelled from both ovaries at the same time; and it is not at all unlikely that some of the eggs originating in the left ovary may pass into the right oviduct and vice versa. Presumably, however, the great majority of eggs from the right ovary pass into the right uterus as, owing to the enormous numbers of eggs that are produced, it does not seem possible that many of the eggs can be moved from one part of the body cavity to the other. The results of this series of experiments, which confirm those previously obtained when batches of eggs from each uterus of a female were fertilized with sperm from the same male (King, '09), show that if two kinds of eggs are produced in the ovaries of *Bufo* they must be produced in approximately equal numbers in each ovary. Experiments similar to these can therefore give no possible clue to the time or to the manner in which sex in *Bufo* is determined.

Hertwig ('07) found that the different lots of frog tadpoles that developed from eggs of the same female that were fertilized with sperm from different males showed marked differences in their growth energy. A similar phenomenon was observed in the various lots of tadpoles of *Bufo* that belonged to this series of experiments. Eggs which were fertilized with sperm from the left testicle of male 2 produced tadpoles which, as a rule, were larger, more uniform in size, and apparently more vigorous than were the individuals in the other lots. The mortality among these individuals was comparatively low. The great majority of the tadpoles that were produced from eggs that were fertilized with sperm from the right testicle of male 3 were somewhat undersized; and they were backward in developing, as only 39 of them had completed metamorphosis by June 22. The mortality in this lot was considerable. Both lots of tadpoles derived from eggs that were fertilized with sperm from the right and from the left testicle of male 1 developed at about the same rate and were of average size. The mortality in these two lots was about 10 per cent.

Owing to a series of accidents a number of individuals in each of these lots were lost; and therefore the comparatively high death rate, as shown in Table II., can be ascribed in great part to this cause. To a similar cause can also be attributed at least 10 per cent. of the mortality in the various other series of experiments described in the present paper.

3. The Effects of Changing the Water Content of the Egg at the Time of Fertilization on the Sex Ratio of *Bufo lentiginosus*.

I. *Extracting Water from an Egg, or Preventing the Absorption of Water by an Egg, during the Fertilization Period.*

If the sex of an embryo is definitely fixed at the time that the egg is fertilized, it is conceivable that the sex-determining process may be of such a character, and so evenly regulated, that placing the egg under conditions that would cause it to lose water or that would prevent its absorption of water during the fertilization period might turn the balance in favor of one sex or the other. Normally, as shown by the investigations of Backman and Runnström ('09), the osmotic pressure of the ovarian egg of *Rana*, and presumably also that of *Bufo*, drops from .48° to .045° when the egg has been fertilized but is still unsegmented. This lowering of the osmotic pressure must be due in great part to the absorption of water by the egg as Bialaszewicz ('08) has recently maintained, although the change is ascribed by Backman and Runnström chiefly to an alteration in the condition of the colloids that is induced by fertilization. Any cause that would tend to prevent the normal increase in the water content of the egg at the time of fertilization might, therefore, produce changes that would be far reaching in their results.

In attempting to ascertain whether extracting water from the egg, or preventing the egg from absorbing water during the fertilization period, would produce any alteration in the sex ratio, two different methods of investigation were employed. For convenience in description these two sets of experiments will be designated as series A and series B.

Series A.—While making experiments with the eggs of *Bufo* in the spring of 1909, a pair of toads was placed in an empty aquarium and left undisturbed for several hours. During this time the female laid a small batch of eggs which were found to be segmenting when the toads were removed from the aquarium for use. The eggs which had thus been normally fertilized out of water were placed at once in a dish of tap water and allowed to continue their development. The female that had deposited the eggs was killed and her remaining eggs were used for the temperature experiments summarized in Table II. of a previous paper (King, '10). Many of the eggs "fertilized dry" died during the gastrulation period, and only 44 individuals lived until it was possible to ascertain their sex. Of these 44 individuals 17 were males and 27, or 61.36 per cent. were females. The lot of eggs from the same female that serves as a control for this experiment, since they were fertilized with sperm from the same male, produced 51.39 per cent. of females.

The outcome of this single experiment has little significance, as the number of individuals in which sex was ascertained is too small to give results of value, especially as it is not known how long the eggs had been laid and fertilized before they were found and placed in water. From the condition of the eggs when they were discovered it was evident that they had been laid at least three hours. The possibility of obtaining normal tadpoles from eggs that had been fertilized out of water having been demonstrated by this experiment, a further series of experiments was made this past spring in the following way: Strings of ripe eggs were removed from the uterus of a female and spread out in an empty glass dish. Then, by means of a pipette, a few cubic centimeters of a concentrated solution of spermatozoa were distributed drop by drop over the eggs in as uniform a manner as possible. The dish was then covered with a glass plate and set aside. Four hours later part of the eggs (lot X) were placed in water. An examination of the eggs at his time showed that many of them had segmented in an apparently normal manner and were already in the 4–8 cell stage of development. The remaining eggs (lot Y) were transferred into fresh water at the end of seven hours, when a considerable number of them were in the 32 cell stage. A number of eggs in both lots failed to segment, possibly because the lack of water prevented the free movement of the spermatozoa.

In these experiments the loss of water from the eggs as a result of evaporation was probably very slight, since the thick jelly-like substance surrounding the eggs would serve to protect them during the time that they were kept out of water. The conditions under which fertilization was effected, however, prevented the eggs from absorbing any considerable amount of water during this period. The early development of these eggs was not interfered with by the abnormal conditions under which they had been fertilized, as in both series segmentation was normal and but slightly later than that of the control lot. The mortality among the older embryos was considerable; and somewhat greater in lot Y where the eggs had remained the longer time out of water. The results obtained in this series of experiments are brought together in Table III.

TABLE III.

Eggs Fertilized Dry.

	Total No. Individuals.	No. of Sex Ascertained.	Males.	Females.	Per Cent. Females.
Lot X (4 hrs.)	243	115	45	70	60.86
Lot Y (7 hrs.)	280	72	21	51	70.83
Control	201	140	65	75	53.57

In each case, as the above table shows, a percentage of females was obtained that is considerably higher than that in the control; the higher percentage of females being found among the individuals of lot Y which had developed from the eggs that had remained the longer time out of water. The possible bearing of these results will be considered in connection with the results of the experiments in series B.

Series B.—The second method used to ascertain whether lessening the water content of the egg at the time of fertilization would produce any alteration in the sex ratio was suggested to me by Dr. E. G. Conklin, and consisted in subjecting the unfertilized eggs to the action of salt or of sugar solutions. These solutions, being hypertonic, would tend to extract water from the eggs or to check the absorption of water by the eggs. To attempt the fertilization of eggs in these solutions would be futile, since Loeb ('92) has shown that the addition of even 2 per cent. NaCl to sea-water anæsthetizes the spermatozoa of *Arbacia*, and a much weaker solution would probably have a similar effect on the spermatozoa of amphibians.

Several preliminary series of experiments made during the spring of 1909 were complete failures, since either the eggs were not fertilized at all after being subjected to the action of solutions of different strengths for varying lengths of time, or practically all of the eggs that segmented died during the early stages of development. The unfertilized eggs of *Bufo*, as well as those of *Rana* according to the investigations of Hertwig ('95), are very sensitive to the action of hypertonic solutions. It was necessary, therefore, to make other experiments this past spring in order to ascertain the maximum strength of solutions that could be employed and the length of time they could act without injuring the eggs beyond the possibility of their being fertilized and continuing their normal development. The experiments from which results were finally obtained were made as follows: a batch of ripe eggs was transferred directly from the uterus of a female into a 2.5 per cent. solution of c.p. cane sugar and allowed to remain there for ten minutes. During this time the eggs were kept in constant motion, either by stirring them with a glass rod or by gently shaking the dish. The eggs were then placed in a small amount of tap water containing a quantity of spermatozoa. A second lot of eggs from the same female were subjected to the action of a 2.5 per cent. solution of c.p. sodium chloride for the same length of time and were then fertilized with spermatozoa from the male used in the previous case.

Even with the weak solution that was used and for the short time that it acted many of the eggs that had been put in the sugar solution were killed, or at least rendered incapable of being fertilized; not more than one half of the eggs subjected to the action of the NaCl segmented, and many of these did not go beyond the gastrulation stage. Morgan ('06) has shown that the fertilized eggs of the frog can develop normally in a 6 per cent. solution of cane sugar, but that the maximum strength of NaCl that will permit of normal development is not much above 2 per cent. The more injurious action of the NaCl on the eggs of the frog, as well as on those of the toad, is doubtless due, in great part, to the fact that its osmotic pressure is several times greater than that of sugar.

As the unfertilized eggs of *Bufo* are so quickly injured by solutions of salt and of sugar it is difficult to decide what effects these solutions could have had in the short time the eggs remained in them. Presumably a slight amount of water was extracted from the eggs by the salt solution; but, as the osmotic pressure of sugar is so low, it is very probable that the chief action of the sugar solution would be to prevent the eggs from absorbing water during the fertilization period.

The mortality among the tadpoles reared from the eggs that had been subjected to the action of the salt solution was much greater than when a sugar solution had been used. In both cases the greatest mortality occurred during the first fortnight after the experiment began. The embryos that lived beyond this time seemed for the most part to have recovered from any injurious effects of the treatment to which the eggs had been subjected before fertilization. In their later development the tadpoles in both lots appeared to be perfectly normal and of average size. None of them showed any specific abnormalities such as those Hertwig ('95), Gurwitsch ('95, '96) and Morgan ('03) produced by subjecting the fertilized eggs of the frog or of the toad to the action of solutions of various salts.

The eggs used in these experiments were taken from the left uterus of the female whose eggs, from the right uterus, were employed in the experiments described in section 2; and they were fertilized with spermatozoa from the male designated as no. 2 in those experiments. The sex ratio in the lot of individuals, belonging to the former series, that developed from eggs that were fertilized with spermatozoa from the same male serve as a control for the present experiments (Table II.).

Table IV. shows the results obtained in this investigation.

TABLE IV.

Eggs Treated with Hypertonic Solutions Before Fertilization.

	Total No. Individuals.	No. Sex Ascertained.	Males.	Females.	Per Cent. Females.
Salt (2.5 per cent.)	264	91	25	66	72.52
Sugar (2.5 per cent.)	463	130	39	91	70.00
Control	380	301	143	158	52.49

Both lots, as the above table shows, gave a percentage of females that is considerably higher than that in the control.

The percentage of females among the individuals that developed from the eggs that were subjected to the action of the salt solution before fertilization is greater than that in the lot where a sugar solution had been used. The difference between the two is only 2.52 per cent., however, so it is evident that the sugar solution had practically as great an effect on the eggs as had the salt solution, in spite of the fact that the latter solution has much the higher osmotic pressure.

Although the methods employed to bring about a change in the water contents of the egg at the time of fertilization were very different in the two sets of experiments just described, there is a very striking uniformity in the results. Both series, comprising five lots of eggs from three different females, show a percentage of females much above the upper limit of the range which is apparently normal for the species and which in three instances is nearly 20 per cent. greater than that in the control. These results suggest, although they by no means prove, that extracting water from the egg at the time of fertilization, or preventing the absorption of water by the egg during this period, in some way affects the sex-determining process and tends to the production of relatively more females.

The manner in which the experiments were made, particularly those in series B, seem to preclude the possibility that the spermatozoa and not the eggs could have been affected by the conditions under which fertilization occurred. The results obtained suggest also that the sex-determining mechanism is in the egg and that it can be influenced by external agencies acting during the fertilization period.

II. *The Effects of the Absorption of Water by the Egg at the Time of Fertilization.*

In connection with the experiments just described it seemed worth while to ascertain whether any alteration in the sex ratio of *Bufo* could be induced by subjecting eggs at the time of fertilization to conditions that would tend to cause them to take up more than the normal amount of water. An increase in the amount of water absorbed could presumably be brought about by fertilizing the eggs in solutions of acid or of alkali, since, according to Loeb ('06), both these substances cause eggs to absorb water, the quantity of water taken up increasing with the quantity of acid or of alkali used.

Of the many preliminary experiments made in the spring of 1909, only one gave results that could be considered of any value. In this case one lot of eggs was artificially fertilized in a .01 per cent. solution of hydrochloric acid: another lot of eggs from the same female was fertilized with spermatozoa from the same male in a .01 per cent. solution of ammonium hydrate. In each case the eggs remained in the solution for one half hour, and were then transferred into fresh water. The mortality in both lots of eggs was very high, and was probably at least 50 per cent. No record was made of the number of individuals with which the experiment was started. The results obtained are indicated in Table V.

TABLE V.

EGGS FERTILIZED IN A SOLUTION OF ACID OR OF ALKALI.

Solution.	No. Sex Ascertained.	Males.	Females.	Per Cent. Females.
Acid	124	72	52	41.93
Alkali	126	55	71	54.76
Control	200	94	106	53.00

The percentage of females obtained from the lot of eggs that was fertilized it the acid solution is somewhat low, yet one might attribute it to a chance variation in the sex ratio: the percentage of females obtained in the lot of individuals derived from eggs that were fertilized in the alkaline solution is but slightly greater than that in the control, and therefore calls for no comment. These results afford little evidence that the sex ratio of *Bufo* can be altered by subjecting the eggs to environmental conditions at the time of fertilization that might cause them to absorb a greater amount of water than usual.

Hydrochloric acid is inorganic, and ammonium hydrate is known to have a very injurious action on developing eggs. It was thought, therefore, that possibly different results might be obtained if eggs were fertilized in solutions of other substances. The experiments were repeated on a much larger scale this past year; acetic acid and sodium hydrate being used in making the solutions. Two strengths of each substance were used; a .01 per cent. solution and a .0025 per cent. solution.

In each case eggs and spermatozoa were placed together in the solution and left for one half hour; the dish containing them being kept in constant motion during this time. As the experiment was carried out, therefore, the solutions might have acted on the eggs alone, on the sperm alone, or on both eggs and sperm. After remaining in the solution the given length of time, the eggs were washed in several changes of fresh water and placed in the dishes in which they were to continue their development. The percentage of eggs that failed to develop was about the same in the lots subjected to the action of the acid solutions as where alkaline solutions had been employed; and was greater, as one would expect, where the stronger solutions had been used. It is not possible to say whether the failure of so many of the eggs to segment is to be attributed to the injurious action of the solutions on the eggs, or whether a large proportion of the spermatozoa were rendered incapable of fertilizing the eggs.

For these investigations the eggs of two different females (*a* and *b*) were used. Female *a* was the one from which the eggs were taken that were used in the experiments summarized in Table III., and the control for the former experiments also serves as control for these. Eggs from female *b* were used for the study of the influence of the spermatozoan on the determination of sex in *Bufo*, and also for the experiments in which eggs were subjected to the action of salt and of sugar solutions before fertilization (Table IV.). In the present case eggs from female *b* were fertilized with spermatozoa from both testicles of the male designated as no. 1 in the former investigation (Table II.). The percentage of females that serves as a control in this case was obtained by taking the percentage of females in the total number of individuals in the former experiments that developed from the eggs that were fertilized with spermatozoa from male 1. Only the stronger solutions were used on the eggs taken from female *b*.

The sex ratios obtained in the various lots of individuals derived from eggs that were fertilized in acid solutions are indicated in Table VI.

TABLE VI.

EGGS FERTILIZED IN SOLUTIONS OF ACETIC ACID.

Eggs from	Strength Solution.	Total No. Individuals.	No. Sex Asc.	Males.	Females.	Per Cent. Females.	Per Cent. Females (Control).
Female *a*	Strong (.01 per cent.)	210	79	46	33	41.77	53.57
	Weak (.0025 per cent.)	145	66	42	24	36.30	
Female *b*	Strong (.01 per cent.)	258	71	42	29	40.84	50.28
		613	216	130	86	39.81	

In all cases the number of individuals in which it was possible to ascertain sex was less than one half of that which the lots contained one week after the experiments were started. In spite of this great mortality, due in some cases to accidents, the results are about the same as that obtained where eggs were fertilized in a solution of hydrochloric acid (Table V.). In this series each lot of individuals gave a percentage of females that is considerably lower than that found in the control, and also much below that which appears to be normal for the species. Curiously enough the smallest percentage of females appears in the lot of individuals reared from the eggs of female *a* which had been fertilized in the weaker solution. This result may mean that a solution of the strength employed in this instance is more favorable to the production of males, but more probably it is merely a chance variation in the sex ratio.

The results of this series of experiments are especially interesting and suggestive when compared with those obtained in other cases where eggs from the same females were fertilized under very dissimilar conditions. Eggs from female *a* were fertilized out of water with sperm from the same male used in fertilizing the eggs in this series. In the one case a great excess of females was produced (Table III.); in the other case the percentage of females falls much below normal. Between the two extremes of the series there is a difference of 24.53 per cent. Eggs from female *b* when subjected to the action of a salt or of a sugar solution before fertilization gave in both instances 70.00 per cent. of females; when other eggs from this female are fertilized in acid solutions the percentage of females falls to 40.84 per cent. Between the extremes of the series there is a difference of 31.68 per cent. These variations in the sex ratios are uniform in the different series: they are apparently beyond the limits of normal variations in the sex ratio of *Bufo*, and they are too large to be justly attributed to mere chance variations in the sex ratios of different lots of individuals.

The percentages of females obtained in the series of experiments in which eggs were fertilized in solutions of sodium hydrate are indicated in Table VII.

TABLE VII.

Eggs Fertilized in Solutions of Sodium Hydrate.

Eggs from	Strength Solution.	Total No. Individuals	No. of Sex Asc.	Males.	Females.	Per Cent. Females.	Per Cent. Females (Control).
Female *a*	Strong (.01 per cent.)	175	49	20	29	59.18	53.57
	Weak (.0025 per cent.)	252	98	42	56	56.12	
Female *b*	Strong (.01 per cent.)	215	75	33	42	56.00	50.28
		642	222	95	127	57.11	

In this series the apparent effect produced on the sex ratio by fertilizing eggs in alkaline solutions is the reverse of that which seemingly results when eggs are fertilized in acid solutions, since in each case a percentage of females is found which is somewhat above that in the control lot. The percentages of females obtained show very much less deviation from those of the control than was the case in the acid series; and in no lot is the proportion of females more than 6 per cent. above that of the control. It seems probable, therefore, that these percentages, although somewhat high, have no especial significance, and that they are chance variations in the sex ratio.

The great difference between the results obtained when eggs of *Bufo* are fertilized in acid solutions and those found when eggs are fertilized in solutions of sodium hydrate would seem to indicate that these solutions have no effect whatever on the sex-determining process, since both solutions would presumably affect the eggs in the same way and tend to cause them to absorb water. The rather unusual sex ratios obtained in the acid series would be explained, on this assumption, as due either to chance or to the action of the solution on the spermatozoa. There is, however, another possible explanation that would serve to bring these results in line with those obtained by subjecting eggs to conditions which tend to lessen the water contents during the fertilization period. It is known that egg membranes are more readily permeable to weak acid than to weak alkaline solutions, and as the jelly-like mass that surrounds the unfertilized eggs of *Bufo* is relatively thick, as is also the zona pellucida that incloses each individual egg, it is possible that in these experiments only the acid solutions were able to penetrate the membranes and act on the eggs. That this interpretation of the results is at least plausible is indicated from the sex ratios obtained in the series of experiments about to be described where fertilizing eggs in acid solutions again leads to a seeming increase in the relative proportion of males, while practically normal sex ratios are found where the eggs were fertilized in solutions of NaOH.

In a former study of the effects of temperature on the determination of sex on *Bufo* (King, '10), the results obtained seemed to indicate the possibility that temperature, acting at the time of fertilization, might indirectly influence the sex-determining process; a high temperature favoring the development of females, a low temperature tending to the production of relatively more males. These experiments showed, however, that the temperature at which the eggs are fertilized cannot be the dominating factor in determining sex in *Bufo*, and that at most its influence would be to facilitate or to hinder certain processes taking place in the egg that are concerned with sex-determination.

In the experiments just described the various lots of eggs were fertilized in solutions that were kept at room temperature (20° C.) during the time that they were allowed to act on the eggs. In another series of experiments a batch of eggs from the female whose eggs were used for the alcohol series of experiments (Table I.) was divided into lots of several hundred eggs each. Each lot of eggs was then fertilized with spermatozoa from the same male in a .0050 per cent. solution of acetic acid or of sodium hydrate that was kept at a temperature of 28°, 20° or 11° C. during the half hour that the eggs remained in it. It was hoped that by this means more certain evidence of the action of temperature in determining sex in *Bufo* might be obtained, since a high temperature would probably facilitate the penetration of a solution into the egg and a low temperature would tend to retard it. Any change in the sex ratio greater than that shown in the other series of experiments in which eggs were fertilized in acid or in alkaline solutions might therefore be attributed to the effects of the temperature at which the solution acted.

A small proportion only of the eggs that were used in these various experiments segmented; and, as in other experiments in which eggs were subjected to abnormal conditions at the time of fertilization, many of the tadpoles died during the early growth period. The results obtained in the acid series are brought together in Table VIII.; the percentage of females given as a control being the same as that for the alcohol series (Table I.).

TABLE VIII.

Eggs Fertilized in Acid Solutions at Different Temperatures.

Temp. of Solution.	Total No. Individuals.	No. Sex Asc.	Males.	Females.	Per Cent. Females.	Per Cent. Females (Control).
11° C.	156	35	23	12	34.28	51.41
20° C.	104	69	46	23	33.33	
28° C.	135	73	50	23	31.50	
	395	177	119	58	32.76	

In spite of the comparatively small number of individuals in which sex was ascertained, very uniform sex ratios were found in all the various lots, and in every case the percentage of females falls far below that which is apparently normal for the species. The smallest proportion of females was found in the lot in which the eggs had been fertilized at the highest temperature; and, conversely, the greatest number of females appears where fertilization was effected at a temperature of 11° C. This is the result one would expect if the higher temperature had facilitated the penetration into the egg of a solution that could influence the sex-determining mechanism in such a way as to increase its tendency to produce a male. The difference between the percentages of females in the two lots in which the eggs were fertilized at the extreme temperatures is only 2.78 per cent., however, so it is evident that the temperature at which the solution acted had very little, if any, influence on the results.

No evidence regarding the influence of temperature on the determination of sex in *Bufo* is given by the results of these experiments, and their chief interest lies in the fact that they accord so well with the results of the other investigations in which eggs were fertilized in acid solutions. If we consider the series as a whole and disregard the possible influence of temperature on the results, it is found that in the total of 177 individuals in which sex was ascertained only 58, or 32.76 per cent. are females. This is 18.65 per cent. lower than the percentage of females in the control lot and 7.05 per cent. less than the percentage of females obtained in the former series of experiments where eggs were fertilized in acid solutions at room temperature (Table VI.).

The results of the experiments in which eggs were fertilized in solutions of NaOH that were kept at different temperatures during the period that the solutions acted are shown in Table IX.

TABLE IX.

EGGS FERTILIZED IN ALKALINE SOLUTIONS AT DIFFERENT TEMPERATURES.

Temp. of Solution.	Total No. Individuals.	No. Sex Asc.	Males.	Females.	Per Cent. Females.	Per Cent. Females (Control).
11° C.	144	54	27	27	50.00	
20° C.	150	66	28	38	57.57	51.41
28° C.	255	126	65	61	48.41	
	549	246	120	126	51.21	

In this series also the sex ratios obtained indicate that the temperature at which the solutions acted has no appreciable influence on the results, although here, as in the acid series, a slightly lower percentage of females was obtained where the eggs had been fertilized at the highest temperature.

Both lots of eggs that were fertilized at the extremes of temperature used gave a percentage of females slightly below that of the control and some 8 per cent. lower than that in the lot where the eggs had been fertilized at room temperature. In the latter case the relatively high percentage of females cannot be considered as significant, as it is balanced by the low percentages of females obtained in the other two lots. In the total of 246 individuals in this series in which sex was ascertained 126 were females. The proportion of females in this series is therefore 51.21 per cent., which is remarkably close to that in the control lot (51.41 per cent.).

In none of these experiments does the observed percentage of females differ in any great degree from that of the control. The results therefore agree very well with those of the other series in which eggs were fertilized in solutions of NaOH (Table VII.). Since the fertilization of eggs in solutions of NaOH produce no apparent effect on the sex ratio of *Bufo*, it is evident that either the solutions used were not strong enough to penetrate the egg membranes and act on the egg in the short time in which the eggs remained in them, or that NaOH is not a substance that can act on the egg in such a way as to increase its tendency to produce a male. A further series of experiments will be made in which the eggs are subjected to stronger solutions of NaOH before fertilization. It is probable that the eggs are less readily injured than the spermatozoa by various solutions, and it may be possible to subject them to the action of a comparatively strong solution without serious injury.

Discussion.

The possibility that the relative amount of water in the amphibian egg at the time of fertilization may have some influence in determining sex seems to me to afford a plausible explanation of the unusual sex ratios obtained by Hertwig ('06, '07), and by Kuschakewitsch ('10), in lots of frogs developed from eggs that were overripe when fertilized. In several of his experiments Hertwig obtained from 70–90 per cent. of males; while in one experiment made by Kuschakewitsch, in which the fertilization of a batch of eggs of *Rana esculenta* was delayed for 89 hours, all of the resulting individuals were males. As the mortality in this last lot was only from 4–6 per cent. it is not possible to explain the results as due to selective mortality. Hertwig attributes the great excess of males in these experiments to the "Kernplasmarelation" existing in the eggs at the time that they were fertilized. He believes that young eggs, and also those that are overripe when fertilized, tend to produce males because they are relatively richer in nuclear substance; females, on the other hand, are produced when the egg is relatively poor in nuclear substance at the time that it is fertilized.

It is very probable that the eggs of the frog normally absorb a slight amount of water during their passage down the oviducts and while they were retained in the uteri. If the eggs remain in the uteri two or three days beyond the time that they would normally have been deposited and fertilized it is not improbable that they continue to absorb water during this time. Overripe eggs, therefore, would contain relatively more water than those that were laid at the normal time. The sex ratios found when such eggs are fertilized accord very well with those that I have obtained by fertilizing the eggs of *Bufo* in acid solutions. In both cases it seems probable that the water contents of the eggs at the time they were fertilized was higher than usual; and one is strongly inclined to attribute the great excess of males to this cause.

Experiments made two years ago with the eggs of *Bufo* to ascertain whether delaying fertilization would produce an alteration in the sex ratio (King, '09) gave negative results. The eggs of *Bufo* are not as favorable as are those of *Rana* for experiments of this kind, as it is not possible to delay the deposition of the eggs more than a few hours. In the experiments referred to a female was killed as she was about to deposit her eggs. The body was not opened until seven hours later when the eggs were artificially fertilized. Post-mortem changes had evidently begun as, although the majority of the eggs segmented in a more or less regular manner, many of them failed to develop beyond the gastrulation stage. The sex ratios were practically normal in the lot of individuals in which sex was ascertained. In the light of these recent experiments the negative results that were obtained can perhaps be atributed to the fact that either the eggs were unable to absorb water during the time that they remained in the uteri of the dead female, or that they were not kept a sufficiently long time to enable them to take up enough water to produce any effect on the sex-determining process.

Unfortunately there is one source of error in all of the experiments described in this paper that makes it impossible to draw any positive conclusions from the results obtained, however definite they may appear in some cases. The mortality in the various lots of individuals was very great and only a small percentage of the eggs with which any experiment was started were carried through to metamorphosis and their sex ascertained. The possibility exists, therefore, that the results may be due solely to chance or to selective mortality, and that in no case was the normal sex ratio really changed by subjecting eggs to the influence of various external factors at or before the time of fertilization. There is, however, no evident relation between mortality and sex in tadpoles reared under artificial conditions, as shown by the investigations of Pflüger ('82) and also by my former work. It does not seem probable, therefore, that in certain lots subjected to similar treatment at the time of fertilization more males than females would always die; while in other lots, where the eggs had received different treatment, the mortality would invariably be greater among the females. Practically normal sex ratios were obtained in all of the control experiments, in the alcohol series, and also in that in which lots of eggs from the same female were fertilized with spermatozoa from different males. This uniformity in the results of so many series of experiments makes it even more improbable that mortality was selective in other cases; neither does it seems possible that the unusual sex ratios obtained in some instances could be chance variations.

The results of the various experiments in which eggs were fertilized in solutions of acetic acid are so uniform that they seem to me very suggestive, even if no definite conclusions from them are possible. Altogether a total of seven lots of eggs from four different females were fertilized in acid solutions. In every instance the percentage of females that was obtained was from 10–20 per cent. lower than that which is probably normal for the species. There is apparently something in this series that stands for a tendency to the production of males rather than of females. These results taken in connection with the equally clear-cut results that were obtained in the five experiments in which water was extracted from the egg, or the egg prevented from absorbing water, would seem to indicate that the relative amount of water in the egg at the time of fertilization has some influence in determining sex; an increase in water content tending to produce a male; a lowering of the water content favoring the development of a female.

It is, of course, possible to explain these results in other ways besides attributing them to chance or to selective mortality. On the assumption that the male is responsible for sex-determination in *Bufo* the results of the acid series of experiments can be attributed to the fact that the solution killed more of the female-producing than of the male-producing spermatozoa; why this should occur is not at all clear. The explanation of the relatively high percentages of females among the individuals derived from eggs that were subjected to the action of a salt or of a sugar solution before fertilization must be ascribed, according to this view, to the fact that the solutions rendered the eggs more resistant to the entrance of one kind of spermatozoa than to the other. This would be, in effect, selective fertilization for which at present there is no evidence, unless we accept the case of the pigeon. According to the investigations of Aristotle, of Fluorens ('64) and of Cuénot ('99) the first of the two eggs laid by the pigeon almost always produces a male. Selective fertilization therefore very probably occurs in the pigeon if the male is responsible for sex in this form.

That the results of any of these experiments are conclusive, I do not for a moment maintain. They seem to me to be unusual enough, however, to warrant the continuation of investigations along these lines. As they stand the results strongly suggest that sex in *Bufo* is determined at or near the time of fertilization, and that external factors acting during this period may influence the sex-determining mechanism in such a way as to cause it to produce one sex or the other. The results also seem to indicate that in *Bufo* sex is determined in the egg, and that it may depend in some way on the relative amount of water in the egg at the time of fertilization. This suggestion, if capable of more general application, does not exclude the possibility that in some cases the spermatozoan may influence sex. It is known that the unfertilized eggs of the bee produce only males, and that fertilized eggs almost invariably develop into females. In this case the entrance of the spermatozoan changes the sex, although, strictly speaking, it cannot be said to determine sex, since the egg has a sex mechanism and is capable of developing without fertilization into a male. If the eggs of the vertebrates could develop parthenogenetically it is very unlikely that the embryos would be without sex. It seems probable, therefore, that in all eggs there exists a sex mechanism that alone is capable of giving sex to the resulting embryo; but in many forms besides the bee the spermatozoan may be a sex-changing factor if not a sex-determining one.

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BIOLOGICAL BULLETIN

EXPERIMENTS WITH CHRYSOMELID BEETLES.

III. THE EFFECTS OF KILLING PARTS OF THE EGGS OF *Leptinotarsa decemlineata*.[1]

ROBERT W. HEGNER.

CONTENTS.

1. Introduction.. 237
2. Killing the germ cell determinants.................................. 240
3. Killing the primordial germ cells................................... 243
4. Killing parts of freshly laid eggs.................................. 244
5. Killing parts of eggs in the blastoderm stage....................... 245
6. Killing parts of young embryos...................................... 247
7. Killing parts of old embryos.. 250
8. Summary... 250

1. INTRODUCTION.

The method used in the experiments described in this paper, *i. e.*, killing parts of the egg, has been employed by a number of investigators in many ways and for many purposes. Heat and electricity are the agents which have been most frequently applied. So far as I know insects' eggs have heretofore never been operated upon in this way. The results obtained by the use of these agents are quite similar to those so frequently brought about by removing parts of the egg or embryo, or by isolating blastomeres. The latter process is of course impossible in the case of the insect's egg, since cleavage is superficial, but material has been removed successfully from different parts of beetles' eggs in various stages of development.[2]

Three years ago a preliminary report was made of experiments in removing portions of the eggs of *Calligrapha multipunctata*,

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[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 131. Parts I. and II. of "Experiments with Chrysomelid Beetles" appeared in the BIOLOGICAL BULLETIN, Vol. XIX., June, 1910, pp. 18–30.

C. bigsbyana, *C. lunata* and *Leptinotarsa decemlineata*.[1] Before the results of those experiments and the data contained in the present contribution can be understood clearly, a brief account of the structure of freshly laid chrysomelid eggs and of the principal stages in their normal embryonic development is necessary.

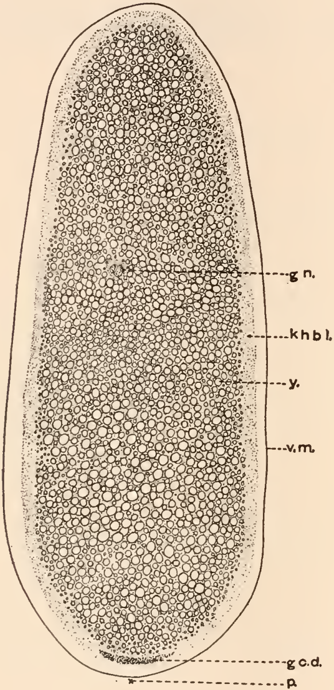

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FIG. 1. A longitudinal section through an egg of *Calligrapha bigsbyana* four hours after deposition. *gcd*, germ cell determinants; *gn*, germ nuclei copulating; *khbl*, "Keimhautblastem"; *p*, posterior; *vm*, vitelline membrane; *y*, yolk. The eggs of *Leptinotarsa decemlineata* are not visibly different from those of *C. bigsbyana*.

The freshly laid egg (Fig. 1) consists of a large central mass of yolk globules (*y*) and a comparatively thin superficial layer of cytoplasm, the "Keimhautblastem" (*khbl*). Two envelopes cover the egg, the vitelline membrane (*vm*) and the chorion. Polar bodies are usually present at this time, and the egg nucleus is in the act of union with the sperm nucleus (*gn*), or else cleavage has already begun. Embedded in the "Keimhautblastem" at the posterior end of the egg is a disc-shaped mass of darkly staining granules, which I have called the pole-disc, or germ cell determinants (*gcd*).

As cleavage progresses, a separation of the cleavage products into sections occurs; the nuclei of one group form a more or less regular layer equidistant from the periphery, and later migrate outward, fuse with the "Keimhautblastem" and become the blastoderm (Fig. 2, *bl*). The nuclei of the other group remain behind in the yolk (Fig. 2, *v*), which it is their duty to dissolve. Eight of the cleavage products which reach the posterior end do not help to form the blastoderm, but gather the germ cell determinants about them and continue their migration until they are entirely separated from the egg; these are the primordial germ cells (Fig. 2, *pgc*).

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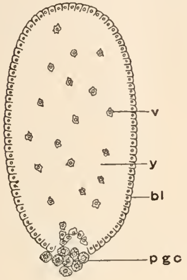
[1] Hegner, R. W., '08*b*, "The Effects of Removing the Germ Cell Determinants from the Eggs of Some Chrysomelid Beetles," BIOL. BULL., Vol. 16



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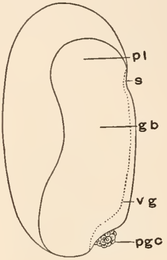

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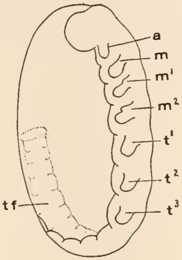

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FIG. 2. A longitudinal section through an egg of *Leptinotarsa decemlineata* one day after deposition when in the blastoderm stage. *bl*, blastoderm; *pgc*, primordial germ cells; *v*, vitellophag; *y*, yolk.

FIG. 3. Superficial view of the right side of an egg of *Leptinotarsa decemlineata* thirty-six hours after deposition. *gb*, germ band; *pgc*, primordial germ cells; *pl*, procephalic lobes; *s*, stomodeum; *vg*, ventral groove.

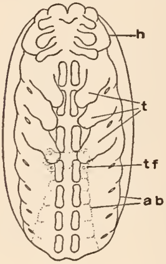
FIG. 4. Surface view of right side of an egg of *Leptinotarsa decemlineata* forty-eight hours after deposition. *a*, antenna; *m*, mandible; *m'*, first maxilla; *m²*, second maxilla; *t'–t³*, thoracic appendages; *tf*, tail fold.

They remain quiescent for a considerable period and then migrate into the embryo, which has developed in the meantime.

At the end of two days the germ band appears on the ventral surface of the egg (Fig. 3, *gb*); this lengthens within the next twenty-four hours, growing forward to the extreme anterior end, and posteriorly until the tail-fold reaches over half way up on the dorsal surface (Fig. 4, *tf*). During this elongation the embryo segments and the appendages of the head and thorax grow out. The entire embryo then contracts antero-posteriorly and begins to grow around the yolk (Fig. 5). This contraction continues until the end of the tail-fold coincides with the posterior end of the egg (Fig. 6). The larva (Fig. 7) usually appears in five or six days.[1]

2. Killing the Germ Cell Determinants.

The method used in the experiments mentioned above to remove the germ cell determinants was to prick the posterior end of the freshly laid egg with a needle and allow them to flow out. These experiments were not considered entirely successful, since I was unable to determine in any case whether all of the germ cell determinants had been removed. Some of the embryos which developed from eggs operated upon in this way produced either a lesser number of germ cells than normal, or else no germ cells at all. The conclusion was reached that if all of the germ cell determinants are removed from the egg no germ cells will be produced, and that the granules which constitute the pole disc are really germ cell determinants.[2]



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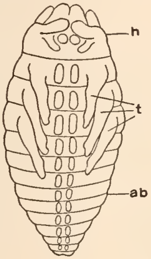

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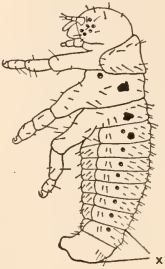

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Fig. 5. Ventral view of an egg of *Leptinotarsa decemlineata* sixty hours after deposition. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold.

Fig. 6. Ventral view of an egg of *Leptinotarsa decemlineata* seventy-two hours after deposition. *ab*, abdomen; *h*, head; *t*, thoracic appendages.

Fig. 7. Side view of a newly hatched larva of *Leptinotarsa decemlineata*.

Two series of experiments (L.D. 09 and L.D. 018) were carried out in order to learn whether or not germ cells would appear in embryos if the germ cell determinants were prevented from taking part in their development. Certain data regarding these experiments are listed in Tables I. and II. Eggs were oriented as soon as laid and the central region of the posterior end of each was touched with a hot needle, thus killing the protoplasm just beneath in which the germ cell determinants were embedded. Since it was impossible to see the germ cell determinants in the living egg, a rather large area had to be killed in order to be certain that all of them had been reached. As noted above, the experiments in removing the germ cell determinants by pricking the egg and allowing them to flow out were not entirely successful because it was never possible to tell whether all of them had been obtained.

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[1] Hegner, R. W., '08*a*, "Observations on the Breeding Habits of Three Chrysomelid Beetles, *Calligrapha bigsbyana*, *C. multipunctata* and *C. lunata*, *Psyche* Vol. 15.

[2] For a discussion of this subject see Hegner, R. W., '11, "The Germ Cell Determinants in the Eggs of Chrysomelid Beetles," *Science*, Vol. XXXIII., pp. 71–72.

TABLE I.

EXPERIMENTS IN KILLING THE GERM CELL DETERMINANTS.

Leptinotarsa decemlineata—Series L.D. 09.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 09 A	Control.			Hatched in 6 days.
L.D. 09 B1	Immediately after deposition (see Fig. 1).	Posterior end killed with hot needle.	0	4 cleavage nuclei present.
L.D. 09 B2			1 day.	See Fig. 8.
L.D. 09 B3			2 days.	
L.D. 09 B4			3 days.	See Fig. 11.
L.D. 09 B5			4 days.	
L.D. 09 B6			5 days.	
L.D. 09 B7			7 days.	

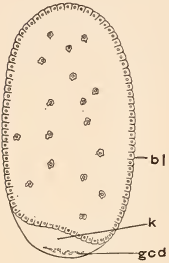
TABLE II.

EXPERIMENTS IN KILLING THE GERM CELL DETERMINANTS.

Leptinotarsa decemlineata—Series L.D. 018.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 018 A	Control			Hatched normally.
L.D. 018 B1	Immediately after deposition (see Fig. 1).	Posterior end killed with hot needle.	0	2 cleavage nuclei found.
L.D. 018 B2			1 day.	
L.D. 018 B3			2½ days.	See Fig. 9.
L.D. 018 B4			3 days.	
L.D. 018 B5			4 days.	
L.D. 018 B6			6 days.	

Many of the eggs operated upon in experiments L.D. 09 and L.D. 018 did not develop at all or developed abnormally. Under ordinary circumstances eggs sometimes become shapeless masses of tissue or remain in the condition of the freshly laid egg, but this fact does not explain the great number of cases observed among the operated eggs. It is evident that many of the operated eggs failed to develop because of the conditions of the experiments. For this reason only a few of the best defined embryos have been selected for descriptive purposes.



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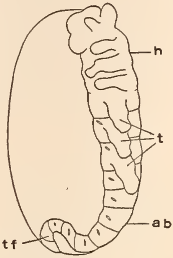

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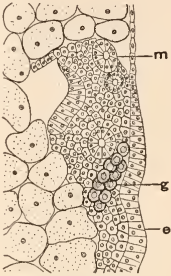

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Fig. 8. Longitudinal secion through an egg of *Leptinotarsa decemlineata* (L.D. 09 B2). The posterior end was killed with a hot needle just after the egg was laid (see Fig. 1); the egg was then allowed to develop for twenty-four hours. *bl*, blastoderm; *gcd*, germ cell determinants; *k*, portion of egg killed.

Fig. 9. Side view of an egg of *Leptinotarsa decemlineata* (L.D. 018 B3). The posterior end was killed with a hot needle just after deposition (see Fig. 1); the egg was then allowed to develop for sixty hours. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold.

Fig. 10. Longitudinal section through the tail fold of a normal embryo of *Leptinotarsa decemlineata* sixty hours old, showing the germ cells (*gc*). *ec*, ectoderm; *m*, malpighian tubules.

Fig. 8 is from a longitudinal section of an egg (L.D. 09 B2) which was operated upon just after deposition and was then allowed to develop for twenty-four hours. This should be compared with the normal egg at a similar stage of development (Fig. 2). In the egg shown in Fig. 8 the germ cell determinants (*gcd*) and a portion of the neighboring yolk and cytoplasm (*k*) have been prevented from taking part in development. That part of the egg which remained alive produced a blastoderm of a single layer of cells (*bl*). The principal difference to be noted between this preparation and that of a normal embryo is the absence of the primordial germ cells at the posterior end (see Fig. 2, *pgc*). Three perfect series of sections were cut from eggs at this stage, but none disclosed any germ cells.

Fig. 9 is a sketch of embryo L.D. 018 B3 (see Table II.). It was killed two and one half days after the operation and is a stage a trifle younger than that shown in Fig. 5. The tail fold (*tf*), however, is not fully developed as in Fig. 5. Longitudinal sections were cut through two embryos like that shown in Fig. 9, but no germ cells could be found. Fig. 10 was drawn from a section through the tail fold of a normal embryo two and one half days old; it indicates where the germ cells (*gc*) are situated at this time. If germ cells had been present in the embryo shown in Fig. 9, they would certainly have been found.

These experiments demonstrate that germ cells are not produced when the extreme posterior end of the egg is prevented from taking part in development, and it seems probable from the method of origin of the germ cells that the destruction of the germ cell determinants is the real cause of their absence.[1]

3. Killing the Primordial Germ Cells.

Table III. gives the data for the experiments performed in series L.D. 011. Sixty-nine eggs were laid at 11 A.M. June 24,

TABLE III.

Experiments in Killing the Primordial Germ Cells.

Leptinotarsa decemlineata—Series L.D. 011.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 011 A	Control.			Hatched normally.
L.D. 011 B1	One day after deposition (see Fig. 2).	Posterior end killed with hot needle.	0	Like Fig. 2.
L.D. 011 B2			1 day.	
L.D. 011 B3			2 days.	
L.D. 011 B4			3 days.	
L.D. 011 B5			4 days.	
L.D. 011 B6			5 days.	One hatched.
L.D. 011 B7			6 days.	Several hatching.

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[1] The writer's papers on "The Origin and Early History of the Germ Cells in some Chrysomelid Beetles" (*Journ. Morph.*, Vol. 20) and on "Germ Cell Determinants and Their Significance" now in press (*American Naturalist*) discuss these points fully.

and were allowed to develop until 11 A.M. June 25. The posterior end of sixty-five of them was then killed with a hot needle. The four controls hatched on June 29. The eggs when operated upon were in a stage like that shown in Fig. 2. One of the operated eggs hatched on June 30 (L.D. 011 B6), several were hatching on the following day, and a number of others were ready to hatch at that time. The one that hatched and three of those ready to hatch were examined and then sectioned and stained. Superficially they resembled the larva shown in Fig. 7, the only difference being the absence of the last two posterior segments which are indicated by the letter *x* in Fig. 7; one possessed all but the last segment. The sections showed that none of these larvæ contained germ cells.

It is evident from these experiments that the primordial germ cells were killed by the operation and no new ones were produced by the developing embryos. This is, I believe, the earliest stage at which surgical castration has been performed among the Insecta. The influence of this operation upon secondary sexual characters could not be determined, since none of these characters make their appearance in the larvæ.

4. Killing Parts of Freshly Laid Eggs.

The experiments described under heading 2, "Killing the Germ Cell Determinants," might be included in this part of the paper, but they were considered of sufficient importance to warrant a special account. In performing the experiments in series L.D. 09 (Table I.) it was found impossible to regulate with any degree of exactness the amount of the egg killed, and, since it was absolutely necessary that all of the germ cell determinants be killed, a larger portion of the egg was killed than desired. Some of these eggs, however, when allowed to continue their development, provided data with regard to the effects of killing a large part of the posterior end of freshly laid eggs.

Fig. 11 was drawn from an egg from series L.D. 09 B4. The portion killed by the operation is labelled *k*; the material that remained alive produced the head (*h*) and part of the thorax (*t*) of an embryo. These parts resemble the corresponding parts of a normal embryo at a like age (see Fig. 6). Apparently that part of the "Keimhautblastem" which remained alive after the operation, became supplied with nuclei, was broken up into cells, and proceeded to develop that particular part of the embryo to which it would have given rise if the rest of the egg had not been killed.

Two other series of experiments, L.D. 07 and L.D. 08, were performed to test these results and in every case the living part of the egg developed as though the entire egg were intact. None of the tissue that is normally produced by the killed portion was regenerated by the living material. The embryos developed up to the time of hatching, but were unable to break out of the chorion.

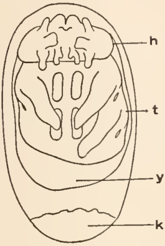

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FIG. 11. Ventral view of an egg of *Leptinotarsa decemlineata* (L.D. 09 B4). The posterior end (*k*) was killed just after deposition (see Fig. 1); a normal head (*h*) and part of the thorax (*t*) developed from the material which remained alive. *y*, yolk.

5. Killing Parts of Eggs in the Blastoderm Stage.

The eggs used for the experiments designated as series L.D. 04 (Table IV.) were laid at 4 P.M. on June 16, and operated upon at 4 P.M. June 17. They were, at the time of the operation, in a stage similar to that of the egg shown in Fig. 2. As indicated in Table IV., two kinds of operations were performed; the anterior part of one half of the eggs was killed with a hot needle, and the posterior part of the other half was killed in a like manner. Three preparations have been selected to show the results of these experiments, (1) L.D. 04 A2, Fig. 12, (2) L.D. 04 A3, Fig. 13, and (3) L.D. 04 B3, Fig. 14.

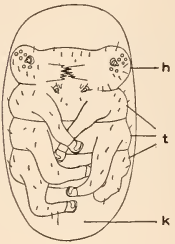
The embryos shown in Figs. 12 and 13 developed from eggs which had their posterior parts killed, and were fixed four days and seven days later respectively. One of these embryos (Fig. 12) consists of a head and thorax which appear to be normal in every respect, and are as fully developed as these parts in a normal embryo at a similar age (five days). The abdomen of this

TABLE IV.

Experiments in Killing Parts of Eggs in the Blastoderm Stage.
Leptinotarsa decemlineata—Series L.D. 04.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 04 A1	Control.			Hatched normally.
L.D. 04 A2	Blastoderm stage (see Fig. 2).	Posterior part killed.	4 days.	See Fig. 12.
L.D. 04 A3			7 days.	See Fig. 13.
L.D. 04 B1		Anterior part killed.	0	
L.D. 04 B2			3 days.	
L.D. 04 B3			4 days.	See Fig. 14.
L.D. 04 B4			7 days.	

embryo is entirely missing. The conclusion is reached that the part of the blastoderm which would have produced the abdomen of the embryo was killed in the operation, and that none of this region was regenerated by the tissue which remained alive.



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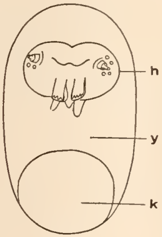

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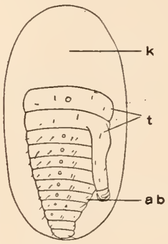

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Fig. 12. Ventral view of an egg of *Leptinotarsa decemlineata* five days old (L.D. 04 A2). The posterior end of the egg (*k*) was killed when in the blastoderm stage (see Fig. 2); the blastoderm which remained alive produced a normal head (*h*) and thorax (*t*).

Fig. 13. Ventral view of an egg of *Leptinotarsa decemlineata* eight days old (L.D. 04 A3), operated upon as in Fig. 12. Only a head (*h*) developed from the tissue which remained alive.

Fig. 14. Side view of an egg of *Leptinotarsa decemlineata* five days old (L.D. 04 B3). The anterior end of the egg (*k*) was killed when in the blastoderm stage (see Fig. 2); the blastoderm which remained alive produced a normal abdomen (*ab*) and part of the thorax (*t*).

Fig. 13 was drawn from an egg that was fixed three days later than that of Fig. 12, *i. e.*, at the age of eight days. Only the head of this embryo developed. It is apparent that not only the tissue destined to produce the abdomen, but also that set aside to form the thorax was killed by the operation. The region of the blastoderm which normally develops into the head covers a considerable area at the time the operation was performed. This area lessens in extent when the germ band arises (Fig. 3, *pl*), and, after the cephalic appendages appear (Fig, 4, *a*, *m*, m^1, m^2), the anterior end of the embryo shortens until the mouth parts are closely crowded together (Figs. 5, 6 and 7). In egg L.D. 04 A3 (Fig. 13) the shortening of the embryo has resulted in the uncovering of a large yolk area (*y*), and, though a comparatively small part of the egg was killed (*k*), this portion bore the blastoderm which in normal eggs gives rise to the larger part of the embryo, *i. e.*, the thorax and abdomen.

When the blastoderm surrounding the anterior end of the egg is killed, only the posterior embryonic region develops from the part which remains alive. This is shown in egg L.D. 04 B3, Fig. 14. Here the normal number of abdominal segments appear as well as two thoracic segments (*t*), one of which has developed a normal pair of legs.

6. Killing Parts of Young Embryos.

The series of figures numbered 15 to 18 show what takes place when parts of young embryos are killed and are thus prevented from continuing development. Table V. gives the data of the operations. The eggs, fifty-two in number, were laid at 10 A.M. June 26; the operations were performed at 10 A.M. June 28, at which time the eggs bore embryos similar to that shown in Fig. 4. Four of the eggs were kept as controls; these hatched on July 2. Approximately one half of the anterior end of twenty-four of the eggs was killed with a hot needle; the posterior half of the other twenty-four eggs was killed in like manner. In every instance the part of the embryo which remained alive developed as though the egg had not been disturbed.

Figs. 15 and 16 show two stages in the development of the posterior part of the embryo, and Figs. 17 and 18 show corresponding stages in the development of the anterior part of the embryo. It is interesting to note that in the egg shown in Fig. 15 not only the anterior end of the embryo, but also the extreme

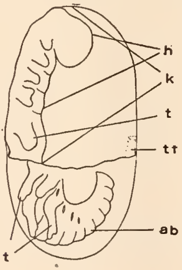
TABLE V.

EXPERIMENTS IN KILLING PARTS OF YOUNG EMBRYOS.

Leptinotarsa decemlineata—Series L.D. 016.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 016 A	Control.			Hatched normally.
L.D. 016 B1	Young embryo (see Fig. 4).	Anterior part killed.	0	See Fig. 4.
L.D. 016 B2			1 day.	See Fig. 15.
L.D. 016 B3			2 days.	
L.D. 016 B4			3 days.	
L.D. 016 B5			4 days.	See Fig. 16.
L.D. 016 C1		Posterior part killed.	1 day.	See Fig. 17.
L.D. 016 C2			2 days.	See Fig. 18.
L.D. 016 C3			3 days.	
L.D. 016 C4			4 days.	

end of the tail fold (*tf*) was killed by the operation, and that the intermediate region consisting of two thoracic segments and eight abdominal segments continued to develop normally except for the mechanical difficulties interposed by the killed material.



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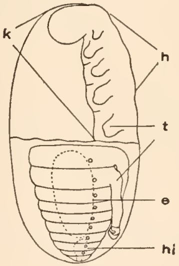

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FIG. 15. Side view of an egg of *Leptinotarsa decemlineata* three days old. (L.D. 016 B2). The anterior end (*k*) was killed when the embryo had reached the stage shown in Fig. 4; the posterior end continued to develop. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold.

FIG. 16. As in Fig. 15, six days old (L.D. 016 B5). *e*, enteron; *hi*, hind intestine.

An *in toto* preparation of an older embryo from this material (Fig. 16) shows that the living part of the embryo succeeded in growing around the yolk and developing a hind intestine (*hi*) which grew forward toward the yolk-filled enteron (*e*).

The preparation shown in Fig. 17 is from an egg fixed one day after the posterior end had been killed, and is the same age as that of Fig. 15, *i. e.*, three days old. It is of special interest, since the end of the tail fold (*tf*), which was not killed by the operation, has continued to develop, although it is an extremely small piece of tissue and was separated from the rest of the living embryo by a considerable amount of yolk. The anterior part of the embryo consisting of the cephalic region and the first thoracic segment, developed normally. During the twenty-four hours between the operation and fixation, the living part of the embryo contracted and left a large yolk space (*y*) between it and the killed material (*k*). A similar condition was noted above in series L.D. 04 A3, Fig. 13 (*y*).

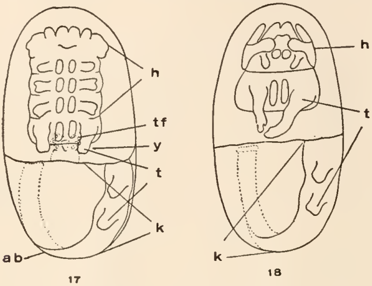

FIG. 17. Side view of an egg of *Leptinotarsa decemlineata* three days old (L.D. 016 C1). The posterior end (*k*) was killed when the embryo had reached the stage shown in Fig. 4; the anterior end continued to develop and has come to lie on the right side of the egg. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold; *y*, *yolk*.

FIG. 18. As in Fig. 17 four days old (L.D. 016 C2).

Fig. 18 represents an embryo (L.D. 016 C2) which was allowed to live one day longer than that just described. Here the head and first thoracic segment have continued to develop reaching a stage similar to that shown in Fig. 6. This part of the embryo has changed its orientation since the operation and now lies on the right side of the egg instead of on the ventral surface. Several other cases like this were observed in series L.D. 016 and in a number of the embryos from other series of experiments.

The last egg selected from this series was fixed three days after the operation at an age of five days. It indicates that development of the living part of the embryo proceeds up to the time of hatching.

7. Killing Parts of an Old Embryo.

The eggs used for these experiments were laid at 4 P.M. June 17, and operated upon at 4 P.M. June 20, at the age of three days. Part of them were kept as controls; the rest were divided into two lots and operated upon as indicated in Table VI. The control eggs hatched on June 22.

TABLE VI.

Experiments in Killing Parts of Old Embryos.

Leptinotarsa decemlineata—Series L.D. 06.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 06 A	Control			Hatched normally.
L.D. 06 B1	Old embryo (see Fig. 6).	Posterior end killed.	0	See Fig. 6.
L.D. 06 B2			1 day.	
L.D. 06 B3			2 days.	
L.D. 06 B4			4 days.	
L.D. 06 C1		Anterior end killed.	1 day.	
L.D. 06 C2			2 days.	
L.D. 06 C3			4 days.	

Fig. 6 shows a normal embryo fixed at the time of the operation. The eggs under experimentation were fixed at intervals and stained and mounted. In every case the part of the embryo that remained alive continued to develop. This was true for both those with the anterior part and those with the posterior part killed. There were no signs of regeneration even after the normal embryonic period had passed. The killed part of the embryo began to disintegrate immediately after the operation.

8. Summary.

1. If the region of a freshly laid egg of *Leptinotarsa decemlineata*, which contains the germ cell determinants (Fig. 1, *gcd*), is killed with a hot needle and these granules are thus prevented from taking part in embryonic development, the embryo produced by the rest of the egg lacks the germ cells. This supple-

ments former experiments in removing the germ cell determinants, and indicates that these granules really determine the germ cells.

2. When the primordial germ cells of *Leptinotarsa decemlineata* are killed in the blastoderm stage (Fig. 2, *pgc*) the resulting embryos lack germ cells. This is the earliest known stage in which surgical castration has been performed among the Insecta.

3. When the anterior or posterior parts of freshly laid eggs (Fig. 1) are killed, the material remaining alive develops that part of the embryo which it would have produced if the eggs had remained intact (Fig. 11). No regeneration of the part which would have been produced by the killed region takes place.

4. If the anterior or posterior parts of eggs in the blastoderm stage (Fig. 2) are killed, the resulting tissue represents the parts of the embryos which would have been produced by the living material if the entire egg had been allowed to develop (Figs. 8 and 9).

5. When parts of young embryos (Fig. 4) are killed, the remaining tissue develops normally (Figs. 15–18). Even small pieces of tissue (Fig. 17, *tf*), which are widely separated from the rest of the embryo, continue to develop normally.

6. Parts of old embryos develop up to the time of hatching. There is no regeneration of the killed part by the living tissue.

7. The eggs of *Leptinotarsa decemlineata*, at the time of deposition (Fig. 1), are definitely oriented with respect to the future position of the embryo.[1] The areas of the peripheral layer of cytoplasm (Fig. 1, *khbl*) are already set aside for the production of particular parts of the embryo, and if these areas are killed, the parts of the embryo to which they were destined to give rise will not appear. Likewise areas of the blastoderm (Fig. 2, *bl*) are destined to produce certain particular parts of the embryo.

THE UNIVERSITY OF MICHIGAN,
February 6, 1911.

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[1] Hegner, R. W., '09, "The Effects of Centrifugal Force upon the Eggs of Some Chrysomelid Beetles." *Journ. Exp. Zoöl.* Vol. 6.

FURTHER EXPERIMENTS ON THE METHODS OF EGG-LAYING IN AMPHITRITE.

JOHN W. SCOTT.

INTRODUCTION.

On excursions, taken in connection with the course offered to students studying invertebrate zoölogy at Woods Hole, I have been impressed with the large number of forms not used for investigation. I believe this must be because little is known of their life-history and habits, because many of them are fairly abundant. With this idea in view, I recorded in a recent paper ('09) the results of some observations made upon the egg-laying habits of one of the marine annelids. In the form studied, *Amphitrite*, the germ cells arise in the typical way, *i. e.*, from the cœlomic epithelium. Very early these dehisce into the body cavity and mix with the cœlomic corpuscles. In some annelids as *Nereis*, the eggs escape by a rupture of the body wall. In *Amphitrite*, however, they pass to the exterior through certain nephridia that are highly modified to form gonaducts. In the paper mentioned above it was shown that *Amphitrite* deposit eggs at recurring periods that bear a close relation to the time of spring tides. It was further shown that at the time of oviposition, the body cavity contains floating free, not only the mature eggs and corpuscles but also the younger eggs in various stages of development. A single period of egg-laying occupies from 30–60 minutes, and most all eggs when extruded are in the metaphase of the first polar spindle. A few large, though unripe, eggs always escape especially toward the latter part of the period. In Fig. 1 is shown the comparative size and shape of various bodies found in the cœlome at this time. It will be noticed that the eggs when first set free in the cœlome are smaller than the red blood cells, yet neither cells nor eggs of this size ever escape during oviposition. Indeed, comparatively few of the large unripe eggs pass out through the nephridia. The interesting question then arises, *How are ripe eggs separated from the other cœlomic bodies in the act of oviposition?*

REFERENCES TO PREVIOUS PAPER.

At first an attempt was made to answer this question by studying the general anatomy of *Amphitrite*. In this group, as shown by Meyer, the septa are incomplete or absent, with one exception. The one complete septum, called the diaphragm, is near the anterior end and divides the cœlome into two unequal cavities. Anterior to the diaphragm the nephridia are for excretion only. The posterior nephridia are modified as gonaducts, and it is with these last that we are concerned. Their inner openings are bordered by large folded, or fimbriated, membranes, covered by strong cilia. Each opening connects with a large vascular sac, also ciliated. From these sacs tubes pass to the outer openings of the nephridia which are found in the species studied on segments 6–10 inclusive. From the dissections I came to the conclusion that there was no apparatus for sifting or "straining out" the ripe eggs.

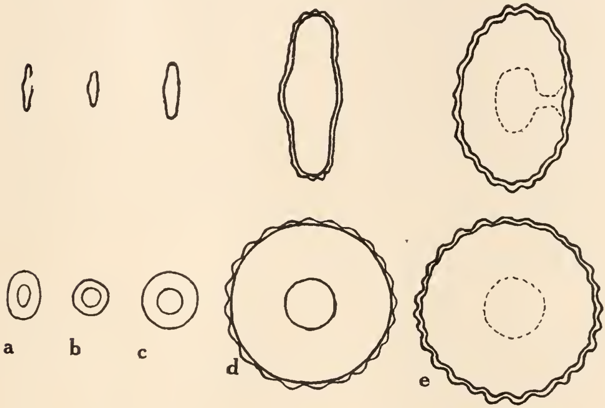

FIG. 1. To show the comparative size and shape of various bodies found in the cœlome of *Amphitrite* at the time of egg-laying; *a*, red blood corpuscles; *b*, youngest free egg observed; *c*, young egg, with little or no yolk; *d*, unripe egg, nearly mature; *e*, mature egg. Both edge and side views shown.

However a theoretical explanation was given in which the nephridia were regarded as "settling basins," the cilia in this case preventing the settling of bodies which were not to be expelled. This explanation was based partly upon the structure of the nephridia, and partly upon the fact that "when the contents of the cœlome are stirred in sea-water, the largest ova including the ripe eggs, always settle more quickly to the bottom of the dish; the immature eggs then settle and last of all the cœlomic corpuscles." The important fact here is that gravity more quickly influences the large ova than the other bodies floating in the cœlomic fluid. It was suggested that the difference in the rate of settling was probably due to a greater density of the ripe eggs, but that the effect was possibly due to a difference in the shape of the bodies concerned. In either case, the conclusion was drawn that gravity is a differential means of separation. However, this left the matter inconclusive and the question arose, *Is this tendency of large ova to settle quickly due to a greater specific gravity, or to a greater mass in proportion to the amount of surface offering resistance?* To answer this was the primary object in the next step of the investigation.

SPECIFIC GRAVITY EXPERIMENTS.

Having set the problem, I began the work of determining the specific gravity of the eggs and corpuscles found in the fluid of the body cavity. Incidentally the specific gravities of sperm, and of eggs in some later stages of development were obtained.

Lyon's plan of getting the density of eggs by centrifuging in gum arabic solution was tried and, after finding it suited my purpose, his method was adopted with slight modifications. First a strong gum arabic solution in sea-water was prepared and its density carefully determined. From this stock solution a series of stock solutions was made up of differing densities; this can be readily done by mixing with the stock solution in proper proportions sea-water of known density. When ready to centrifuge the capillary tubes of a hæmatocrit were filled about three fourths full of gum arabic solution. On top of these solutions whose density was known was placed the material whose density was required. In a few cases, in order to guard against possible error caused by surface tension, the material was placed between two solutions of differing densities and then centrifuged. But surface tension did not appear to offer any appreciable resistance to the passage of the eggs and corpuscles either upward or downward. It was found that eighty turns of the hæmatocrit in one and one half minutes was sufficient to produce thorough separation of materials in the capillary tubes, without noticeably affecting the material within the egg membrane. This amount of centrifuging was therefore adopted throughout the entire series. I shall here present in order my findings in regard to (1) the specific gravity of eggs, (2) the specific gravity of corpuscles, and (3) the specific gravity of sperm, together with remarks as to the significance of these results.

After a number of preliminary experiments, in order to get familiar with the method to be used and to guard against possible sources of error, a series of tests was made upon eggs recently deposited. The results of some of these tests are shown in Table I. The female used in this series was found depositing eggs in a normal manner. Just as quickly as it could be done, while they were still being deposited, these eggs were centrifuged with the results shown in tests 25 to 30 inclusive. The table shows that the specific gravity of recently deposited, that is, mature *Amphitrite* eggs, lies between 1.075 and 1.085. They probably have a mean density very near 1.078; for almost all eggs remain on top of, and therefore are lighter than a solution with a specific gravity of 1.080, while more than half sink in a solution with density at 1.0775. Tests were made on eggs from other worms and verified the results given here.

TABLE I.

To Show the Specific Gravity of Recently Deposited *Amphitrite* Eggs

Test No.	Density of Solution Used.	Material Used.	Result of Centrifuging.
25	1.090	Eggs just deposited.	Eggs on top.
26	1.085	Eggs just deposited.	Eggs on top.
27	1.080	Eggs just deposited.	A few begin to sink.
28	1.075	Eggs just deposited.	All went down.
29	1.0775	Eggs just deposited.	More than one half sink.
30	1.075	Eggs just deposited.	All on bottom.

Another series of tests was made in order to demonstrate the changes in density at different stages of development. See Table II. It was shown that very young eggs, those that had attained one fourth the size of mature eggs and in which there was not very much yolk laid down, had a specific gravity less than 1.070 (Fig. 1, *c*). In a word, the density of the egg as a whole is very noticeably increased as the yolk accumulates. It was also learned that allowing the deposited eggs to stand in sea-water for some time slightly increases their density (test 38). During segmentation and the formation of the segmentation cavity, the density grew less as one might expect (tests 41, 47). It probably continues to decrease slightly, at least as far as the late trochophore stage (test 57). My experiments did not carry the work further than this point.

TABLE II.

To Show the Change in Density at Different Stages in the Development of Eggs.

Test No.	Density of Solution Used.	Material Used.	Results of Centrifuging.
9	1.070	Cœlomic contents. Eggs half-grown and smaller.	Youngest eggs on top.
12	1 075	Cœlomic contents. Eggs nearly mature.	All eggs went down.
13	1.075	Eggs in various stages.	Unripe eggs on top. Ripe eggs down.
27	1.080	Mature eggs, just deposited.	Almost all on top, a few sink.
38	1.080	Same lot, after being in sea-water 1½ hours.	One half of eggs sink.
41	1 080	Same lot, fertilized. 8–16-celled stages.	One fifth of eggs sink.
47	1.075	Same lot, one hour later.	A few eggs sink. Most eggs partly down.
57	1.075	Same lot, trochophores, age 27 hours.	One third trochophores sink Best developed on top.

Before the worm used in the first tests mentioned above, was through depositing eggs, the surface of its body was wiped dry and the cœlome was opened allowing the contents to escape into a clean glass dish. An examination with the microscope showed that the cœlomic fluid contained corpuscles and eggs in various stages of development, some being mature. Tests 32 to 37, Table III., show typical results in regard to the specific gravity of the corpuscles. Upon examining these results one finds that

TABLE III.

To Show the Specific Gravity of Cœlomic Corpuscles.

The cœlomic contents were removed after the worm was about through depositing eggs.

Test No.	Density of Solution Used.	Material Used.	Result of Centrifuging.
32	1.080	Cœlomic corpuscles. Eggs in various stages.	Corpuscles sink. A few ripe eggs sink.
33	1.085	Cœlomic corpuscles. Eggs in various stages.	All corpuscles sink. All eggs on top.
34	1.090	Cœlomic corpuscles. Eggs in various stages.	Seven eighths corpuscles down. Eggs on top.
35	1.095	Cœlomic corpuscles. Eggs in various stages.	Most corpuscles sink. Eggs on top.
36	1.105	Cœlomic corpuscles. Eggs in various stages.	Two thirds corpuscles sink. Eggs on top.
37	1.123	Cœlomic corpuscles. Eggs in various stages.	One tenth corpuscles sink.
46	1.123	After standing three hours in sea-water.	Nearly one third corpuscles sink.

the specific gravity of the cœlomic corpuscles varies between wide limits. However, more than four fifths of them have a density greater than 1.090 and less than 1.123. Contrary to what my previous observations had led me to expect the corpuscles have a density greater than the mature eggs. All corpuscles are heavier and all eggs lighter than a density of 1.085. After standing in sea-water for some time the corpuscles appear to increase in density as shown by test 46, and by other tests not given. Furthermore, the smaller and what appear to be the younger corpuscles have a density less than the older ones. Under these circumstances an examination with the microscope after centrifuging was of course necessary to distinguish between the corpuscles. The significance of these results will be explained later.

In the course of my experiments the cœlomic corpuscles of both males and females were examined. While not suspected at the time, upon looking over my notes the rather curious fact came to light that the mean density of the female corpuscles is slightly greater than that of male corpuscles. I do not wish to emphasize this fact, for perhaps the number of individuals examined was not sufficient on which to base conclusions. Nor yet do I see whether the significance of these results pertains to nutrition alone or to differences in reproduction, should they prove generally true for other annelids.

Another series of experiments showed the extreme lightness of the sperm; their specific gravity in sea-water being between 1.038 and 1.046. In contrast to eggs which increase in density as they mature, the sperm masses decrease in density as they grow larger and finally break up into free-swimming sperm. So that the density of the ripest sperm is even less than 1.038, as shown in Table IV. This density is interesting when considered in connection with fertilization and the habitat in which the animal lives. Water currents are undoubtedly important factors in the dissemination of sperm. But the sand flats on which these animals are found are securely protected from violent currents, where reefs or eel grass or shoals of adjacent islands break the force of the changing tide. The limited range of the animal does not require a wide scattering of the sperm. In fact the density of the sperm is such that, in the presence of very gentle currents, their distribution must be very limited at the time of oviposition. But the lightness of the sperm provides an easy

TABLE IV.

To Show the Specific Gravity of Sperm.

Test No.	Density of Solution Used.	Material Used.	Result of Centrifuging.
8	1.075	Contents of cœlome.	All sperm on top. All corpuscles sink.
40	1.075	Sperm shed in sea-water.	All sperm on top.
42	1.070	Sperm shed in sea-water.	All sperm on top.
43	1.062	Sperm shed in sea-water.	All sperm on top.
44	1.051	Sperm shed in sea-water.	All sperm on top.
45	1.038	Sperm shed in sea-water.	All sperm at bottom.
48	1.046	Sperm shed in sea-water.	All sperm on top.
50	1.041	Cœlomic contents of worm that deposited sperm on previous day.	All unripe sperm sink; a few nearly ripe on top.
52	1.046	Cœlomic contents of worm that deposited sperm on previous day.	Younger sperm masses sink. Older sperm masses on top.
55	1.051	Cœlomic contents of worm that deposited sperm on previous day.	Younger sperm masses sink.
56	1.038	Cœlomic contents of worm that deposited sperm on previous day.	A few lightest, ripest sperm on top.

means for scattering them by currents over the sand flats, and their own locomotion aids them in their distribution. They have sufficient motor power to aid in horizontal distribution, and have sufficient density to prevent them from rising much above the bottom where the eggs are found. That sperm have such a method of distribution was substantiated in a different way by studying the scattering of sperm in dishes of still water. A large quantity of sperm was introduced at some point in the bottom of the dish; then the progress in distribution was observed by noting the advancing cloudiness of the water, reckoned in various directions from the point of departure. It was found that the advance in a general horizontal direction was many times more rapid than the advance in an upward direction. I take this to be due to the resistance of gravity. Since the sperm are set free near the bottom under normal conditions, their lightness tends to bring about more favorable chances for fertilization of the eggs.

We may now sum up the results of these specific gravity experiments in the form of certain explanations and conclusions:

1. The eggs of *Amphitrite* increase in specific gravity during growth in the cœlome. This is probably due to an increase in the amount of yolk. The reason therefore that the larger mature eggs settle in a dish of sea-water more rapidly than the smaller, immature ones, is undoubtedly due in part to the greater specific gravity of the older eggs. And in the process of egg-laying it is probable that a difference in specific density may act as a means to separate ripe from unripe eggs.

2. The reason why the larger eggs sink before the cœlomic corpuscles must be explained in another way, for the specific gravity of the corpuscles is decidedly greater than that of the eggs. The difference in behavior between these bodies is to be explained, I believe, by the flat, oblong shape of the corpuscles; their shape is such that they offer in settling a much larger resistance in proportion to their mass. Blood cells settle more slowly than eggs in sea-water because of a difference in shape and not because of a lesser density. Slight currents in the dish prevent the corpuscles from settling for a long time, while they hardly interfere with the downward movement of the eggs. At this

time in my experiments it was thought probable that ciliary action produces a similar effect in separating eggs and corpuscles during oviposition. This will be discussed later.

3. While the egg increases in density up to the time of oviposition it decreases slightly after fertilization, the decrease continuing at least as far as the trochophore stage.

4. The comparative lightness of the sperm is undoubtedly useful as an adaptation for scattering them at the time of fertilization. Their density is just sufficient to tend to keep them near where the eggs are to be found, but not enough to prevent a certain amount of locomotion.

Direct Observations.

During the past summer I was able to make a series of direct observations upon the separating process. In these observations I saw the ripe eggs pass over the fimbriated membrane, along the grooves, and finally into the vascular, nephridial sac ready to be expelled. At the same time the blood cells and unripe eggs were rejected and thrown back into the cœlomic fluid. The sight was indeed astonishing to see the general precision of a comparatively simple process.

It was by means of living dissections that the observations were made possible. First it is necessary to use only ripe females, and to determine this fact with certainty one needed to wait until the worm began to deposit eggs; then it was lifted out of sea-water and the surface of the body quickly dried. Next the cœlome was opened and its contents carefully drained into a clean watch glass; this was kept covered to prevent unnecessary evaporation while the nephridia were being removed. The worm was then placed in a dry dissecting pan and pinned at either end, so that it was held in a slightly stretched condition. The body cavity was opened by making a cut through the body wall in the mid-dorsal line; this cut began a little back of the middle of the body and extended forward to the anterior end. The enteric canal and accompanying blood vessels were next divided midway and their anterior portions removed. The flaps of the body wall on either side were then pinned out. In order to have these flaps spread out well, and so expose the large nephridial sacs, it was necessary to cut the series of oblique muscles which extend from the body wall above the notopodial cirrus across the cavity to the body wall in the mid-ventral region. This species has five pairs of nephridia that function as oviducts. The cœlomic fluid of a ripe female contains some ripe eggs, many unripe ones, and thousands of corpuscles, mostly red blood cells. Each nephridium to be observed was removed entire and placed at once in the watch glass with the cœlomic fluid. Then the watch glass was placed on the stage of a microscope and the process studied.

As mentioned before the fimbriated membrane is a folded, or grooved structure covered for the most part with strongly developed cilia. The stroke of the cilia varies in direction and force on different parts of the membrane and their action furnishes the motor power that separates the eggs. By using a needle I could push near the fimbriated membrane any of the bodies I wished to study. The cilia are in continuous action, and their effective stroke, so far as I could observe, has a constant general direction. But the direction of the stroke varies on different portions of the membrane as follows: (1) On the edge of the membrane (Fig. 2, *a*) and near the entrance to the grooves between the folds, the stroke is such as to send currents toward or into the grooves. (2) In the grooves (Fig. 2, *b*), where the folds approach somewhat nearer to each other, the stroke at the bottom of the groove is still onward and inward; well up on the sides of the grooves the currents move upward and outward, and apparently with more force than at the bottom of the groove. (3) In the main groove (Fig. 2, *c*) the cilia at the bottom are arranged in approximately parallel lines that resemble small grooves, all leading inward and their action comparatively slow. Above on the sides of the pillars (Fig. 2, *d*), especially in the narrow grooves between pillars, the currents move rapidly and are directed upward and outward. With this explanation of the ciliary currents, both in regard to position and action, we may now consider how the different bodies are separated.

The Separation of Blood Cells from Eggs.—This occurs under the conditions of my experiment principally on the outer edges of the membrane. The currents are strong enough to lift and draw in toward the membrane blood cells from a considerable distance; these cells therefore hit the cilia with some force, but the major stroke of the cilia is strong enough to bounce them off again, usually driving them away five to ten times their own diameter, and frequently beyond the limits of further attraction. However they may bounce two, three, or four times, that is, until they reach the region of the groove shown at *b*, Fig. 2; then the cilia on the sides carry them up, out, and away. Only in very rare instances do blood cells ever reach the main groove; sometimes they follow the eddy near a mature egg, but they are

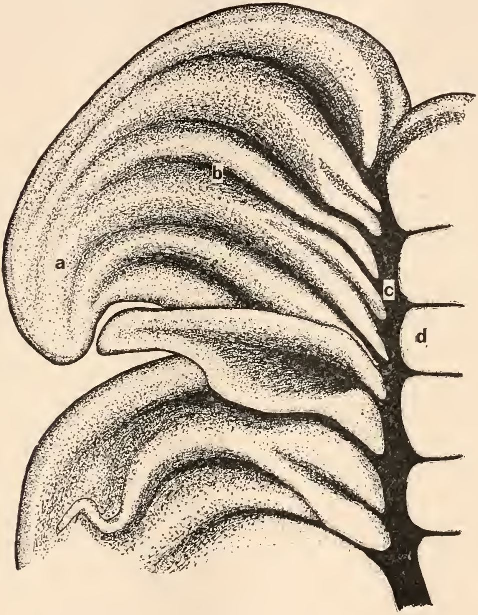

FIG. 2. To show a portion of the fimbriated membrane found at the inner end of a post-diaphragmatic nephridium of *Amphitrite*. Not so much magnified as Fig. 1. The membrane is undulating at *a*, and folding to form a groove at *b*; at *c* is the deep main groove leading into the nephridial sac; at *d*, are pillar-like projections overhanging the main groove. For further explanation see text.

then thrown across the groove and against the pillars where the currents hurriedly lift them up and drive them away. I did not see a blood corpuscle pass down the main groove into the nephridial pouches, so long as the cilia retained their normal activity. After an hour or so their action becomes slower, but this is probably due chiefly to the fact that much plasma has evaporated.

The Separation of Immature from Mature Eggs.—The small, immature eggs, Fig. 1, *b* and *c*, as well as some of a larger size are eliminated in much the same way as blood cells. The task grows more difficult, however, as the eggs increase in size for they become too bulky to be bounced off the edge of the membrane like the blood cells. So, as the larger immature eggs (Fig. 1, *d*) are carried into the narrowing grooves, they are rolled up on edge, then lifted out and moved away. For when the sides of the groove narrow down so that both sides of the flat egg are affected by the currents produced by cilia on the opposite sides of the groove, the combined action of these currents is sufficient to lift even the weight of a large, immature egg. The case is different with the mature eggs, which *differ in shape*, are *more plastic*, and are *somewhat heavier* than most immature eggs found in the cœlome. These eggs are carried into the grooves down which they slowly pass, their shape and weight evidently being such that the lateral cilia are unable to lift them out of the groove. Undoubtedly their plasticity is also an important factor in resisting the action of the cilia. The side of the egg gives before the effective stroke of the cilia, so the result is to change the shape of the ripe egg rather than to push it away, and consequently such eggs move more slowly along the grooves. In some cases it was observed that the folds of membrane along the sides of the groove would respond to the presence of a ripe egg by folding over, virtually forming a closed tube. Inside this tube-like groove the egg could occasionally be seen changing shape, as it was forced slowly along toward the nephridial sac. When the membrane folded it was impossible for unripe eggs, or corpuscles on the membrane, to pass into the nephridial pouch with the ripe egg. The behavior of the membrane in the presence of a ripe egg had all the appearance of a tactile response.

and if any chemical reaction was here involved it could not be detected with the eye. The separation of these bodies appeared to be a purely physical process.

Since the cilia are continuously active, independent of any egg-laying period, it would seem that the egg-laying reaction is a direct response to changes in the egg that are chiefly produced by the breaking down of the germinal vesicle. As noted in an earlier paper a worm shows unusual muscular activity at the time of oviposition. This muscular reaction may be due to chemical changes in the body fluid, the irritation being produced by materials escaping from the nucleus. However another explanation is possible. As the ripened eggs begin to accumulate in the nephridial sacs, excretion, the normal function of these organs, would be hindered or stopped. This would lead to an accumulation of waste in the part of the cœlome posterior to the diaphragm, and in all probability would act as an irritant producing more vigorous muscular action until the eggs were expelled. In this way the wave-like movements of the worm at oviposition are a physiological response to an interference with excretion. Though these suggestions are largely theoretical, I am inclined to believe the latter view is the correct explanation.

Summary and Conclusions.

Amphitrite under normal conditions keeps up, with occasional brief resting periods, a series of wave-like contractions of the body for the purpose of sending water through its tube. A short time before oviposition the wave-like movements become stronger and slightly faster than usual. This excitement indicates the presence of ripe eggs and marks the beginning of the separating process. After some time has elapsed, and the nephridial sacs are well filled, each contraction wave as it approaches the anterior end of the body forces out through the pores small slimy streams of eggs. Oviposition usually continues from thirty to sixty minutes. The process of separation has been previously described. While the cilia furnish the motor power in separating the ripe eggs, strong wave-like contractions of the body wall furnish the pressure needed to expel the eggs from the nephridial pouches. It is suggested that this unusual activity of the worm is probably due to interference with excretion, caused by clogging the nephridial sacs with eggs.

Undoubtedly much the same process occurs in other worms where eggs accumulate in nephridial sacs before oviposition. I am told by Downing that eggs accumulate before oviposition in the nephridial pouches of one species of *Arenicola*. Gerould has reported that the eggs of *Phascolosoma* collect in this way for several hours before they are laid, and there is little doubt that the same general method of egg-laying occurs in all worms with this habit. My results may be summarized in the following conclusions.

1. The separation of cœlomic corpuscles and unripe eggs, at the time of oviposition in *Amphitrite*, is accomplished by physical not chemical means.

2. The work of cilia on the fimbriated membrane furnishes the power used in the separating process. Wave-like contractions of the body aid in expelling the eggs, and it is suggested that these movements are due to interference with excretion, caused by clogging the nephridial sacs with ripe eggs.

3. The separating process is aided in several ways. The *shape* and *arrangement of grooves* on the fimbriated membrane, the *size* and *shape* of the bodies separated, and especially the *greater plasticity* and greater *density* of the mature eggs, all appear to be important factors in separating the bodies found in the cœlome at the time of oviposition. The action of the grooves in closing over a ripe egg is also a direct help in keeping other bodies from the main groove.

4. We are fairly safe in concluding that the method of egg-laying described for *Amphitrite* holds good for other worms where the eggs accumulate in nephridial sacs before or during oviposition.

NEMATOCYSTS OF MICROSTOMA.[1]

WILLIAM A. KEPNER.

For the past three autumns many brown *Hydras* and *Microstomas* were found in a fish pond northeast of the University of Virginia. In both these forms of animals were present oval, refractive bodies, which were the nematocysts. These nematocysts when everted have in both cases slender filaments with three barbules radiating from their base. The filament at its base is attached to the neck of a pear-shaped "poison-sack" or capsule. Since Oersted (1844) nematocysts were known to occur in flatworms as well as in Cœlenterata. Associated with the nematocyst of *Hydra* there is a cell which has elaborated the nematocyst and cares for it until it is discharged. This cell is known as a *cnidoblast* or *nematocyte*. Each cnidoblast of *Hydra* has a small spine-like structure—the cnidocil. This is supposed to receive the stimulus that results in the discharge of the nematocyst. When one examines the living *Hydra* and the living *Microstoma* found in this vicinity no difference can be detected between the nematocysts of the two forms, except that there is no cnidocil associated with the nematocysts of the flatworm. Moreover the nematocysts in both *Hydra* and *Microstoma* when undischarged and when being discharged appear to be normal parts of the respective animals. This indigenous appearance and ready discharge of the nematocysts of *Microstoma* led men to look upon them as structures elaborated by the cells of *Microstoma*. Such was my view after I had repeatedly, during three years, studied the nematocysts of living *Microstoma*. According to Martin (1908) until 1903 all zoölogists held that the nematocysts of *Microstoma* were its own products.

Recently the inference has been made that the nematocysts of *Microstoma* have been derived from ingested cœlenterata, as Grosvenor (1903) had claimed for the nematocysts of æolids. Martin (1908) must be given the credit for having first approached this question of the origin of the nematocysts of *Microstoma* in the proper way, *i. e.*, experimentally.

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[1] The author wishes to thank Professor A. H. Tuttle, of this laboratory, Professor E. G. Conklin and Professor Ulric Dahlgren, of Princeton University, for valuable suggestions that were helpful in the preparation of this paper. This paper was read before the Philosophical Society of the University of Virginia.

Martin first made the observation that *Microstoma* do ingest *Hydras*. "If a fasting *Microstoma* is placed in a watch-glass which contains some small *Hydra*, it is almost certain in a short time to come in contact with one of them. If the *Microstoma* comes suddenly against the tentacles of the *Hydra* it contracts itself immediately, and in this condition it may frequently be killed by the discharged nematocysts. As a rule, however, the *Microstoma* fixes itself for a short time by its posterior end in the neighborhood of the *Hydra*, and everts its pharynx to its full extent.

"The *Microstoma* then swims over the surface of the *Hydra*, usually attacking the lower part of its body with its pharynx fully everted (vide Fig. 10). The *Hydra* then usually becomes strongly contracted, and sweeps its tentacles over to the side on which it has been attacked, though under these conditions the tentacles do not grasp the *Microstoma*, but remain extended almost parallel with its body, and it would appear as though the pharyngeal secretion had a paralyzing action on the *Hydra*. In many cases, after a time, the *Microstoma* leaves its prey, and in such a case the *Hydra* does not seem much the worse for the attack, but if the *Hydra* is of small size, it may be engulfed and swallowed whole" (Martin, 1908, pp. 267–8).

After this observation Martin's chief evidence is based upon the histology of *Microstoma*. He finds that when both *Hydra* and *Cordylophora* are fed to *Microstoma* there are found within the tissues of the flatworm nematocysts peculiar to both kinds of cœlenterata that had been fed. Thus in sections of the *Rhabdocœle* he discovers nematocysts in the endoderm, mesoderm, and ectoderm. Martin's methods of procedure had convinced me that he had established that the nematocysts of *Microstoma* were derived from *Hydra*, until I had considered the details of his work.

I hesitate, for example, to follow Martin when he says that von Graff (1882) was wrong in saying that each nematocyst lies in a single cell and that "Hallez had correctly described the nematocysts as lying in vacuoles" (Martin, 1908, p. 263). It had been the experience of students in my classes to find discharged nematocysts *enclosed within a single cell* (Fig. 1). Such isolated nematocysts were obtained by adding a drop of 1 per cent. acetic acid to a slide containing specimens of living flatworms. Some time after a cell of this kind had lain in the dilute acetic acid a large vesicle appears at the pole from which the discharged nematocyst must emerge (Fig. 2). Such distortion has frequently been observed under similar conditions.

So much for my observations upon the conduct and anatomy of living specimens and upon temporary preparations.

Before taking up the evidence afforded by permanent histological preparations I must interpolate my hesitancy, at least, to follow Martin when he says, "I have found very young *Microstoma* in which nematocysts were already present, and at first this presented a difficulty to this theory of the derivation of nematocysts, but I do not believe this is insuperable when we consider that nematocysts in the case of an animal which has fed largely on *Hydra* can be found in almost any tissue of he body. I have found them in the testes (Fig. 8), and although I have not yet found them in an ovum, I believe that the yolk-cells might readily carry them into the cocoon thus causing the infection of the young forms" (Martin, 1908, p. 271). It does not appeal to my judgment that nematocysts with cnidoblasts should be able to "infect" the young forms through the yolk-cells. Nematocysts *within* vacuoles, without attending cells to propogate them, *infecting* young forms through yolk-cells seem to be altogether improbable.

When I consider this last statement of Martin and note his oversight of the cells that in *Microstoma* do enclose some of the nematocysts I am led to believe that the final word has not been spoken concerning the nematocysts of *Microstoma*.

Such was my attitude when during September, October, November and early December, 1910, collections of *Microstoma* were made with a view to studying their histology.

Many specimens were fixed in chrom-aceto-formal; others were fixed in saturated solution of corrosive sublimate to which 5 per cent. of glacial acetic acid had been added. Both of these fixing fluids gave good results. The sections were mounted serially. Some individuals were cut 3 microns, others at 4, 5, 6 and 7 microns. All the material was stained with Haidenhain's iron hæmatoxylin. Some sections were counter stained with Bordeaux red. From these many individuals a large list of sectioned nematocysts has been made.

As the specimens were being collected it was not an unusual experience to find greatly gorged specimens, which upon the addition of the fixing fluid would burst and thus liberate or expose a dead *Chætogaster* such as is represented in Fig. 3. In passing, this observation is of interest, for Martin says "one of the commonest enemies of *Microstoma* appears to be *Chætogaster*, which devours it greedily" (Martin, 1908, p. 268).

Within the enteron of the sectioned *Microstoma*, one frequently finds the large unbroken setæ of these ingested oligochætes (Fig. 4). If according to Martin, as I interpret him, the passage of the nematocysts from the enteron through endoderm and mesoderm to ectoderm is passive, I wonder why at times these harsh setæ of *Chætogaster* do not passively find their way into the tissues of the body of the flatworm. Other solid bodies such as diatom shells, and round or spheroidal objects such as the shells of *Arcella* lie within the enteron. These too, if the migration of the nematocysts be passive, should find their way to the surface as do the nematocysts; but not a single instance of such objects lying outside the lumen of the enteron was observed.

If, therefore, nematocysts do migrate from the cavity of the enteron to the general surface of the body, their migration seems to be accomplished through some active agency.

The first tissue of course to be concerned in this selective function is the endoderm. The cells of the endoderm must distinguish between the nematocysts and other solid or rounded objects within the enteron, just as an *Amœba* distinguishes between food and non-food. So far as the present series of observations is concerned twenty endodermal cells were found in which a nematocyst was enclosed (Fig. 5). In each case the nematocyst lies within a vacuole near the base of the endodermal cell.

The question at once arises, might not this nematocyst have wandered from the mesoderm into the endoderm? There is ample evidence of nematocysts migrating from one region to another in the bodies of cœlenterata. Schneider says: "Alle Nesselzellen der Siphonophoren enstehen an localisierten Bildungsherden, von denen sie in einen bestimmen Entwickelungstadium als Wanderzellen auf die Verbrauchstätten überwandern" (Boulenger, 1910, p. 764). In this connection "Hadzi's results are of the greatest interest, as he was able to examine living tissue as well as preserved material. His main conclusions are as follows:

"1. The thread-cells of hydroids are not formed 'in situ' but in the ectoderm of the cœnosarcal branches, where, on account of the thick perisarcal investment, they can obviously not become functional.

"2. When completely developed, except for accessory structures such as the cnidocils and the stalks, they migrate to the important nematocyst batteries on the tentacles. This migration can take place in two different manners. In simple forms, *e. g.*, *Campanularia*, the thread cells move actively by means of their pseudopodia, making their way between the ectodermal cells of the colony. In *Tubularia*, however, they adopt a quite different method of locomotion: from the ectoderm of the cœnosarc they force a way through structureless lamella and endoderm into the cavity of the hollow stem, whence they are carried by the current caused by the flagella of the endoderm cells to the hydranths. Here the thread-cells re-enter the tissues and migrate actively by their own movements to the ectoderm of the tentacles" (Boulenger, 1910, p. 764). Conklin (1908) likewise observed the formation of nematocysts within the mesoderm of Actinia and their subsequent migration to the ectoderm. Boulenger states "that in *Mœrisia* the nematocysts of the oral battery of the medusa are developed in the endoderm at the base of the manubrium; this does not necessarily imply that the nematoblasts are themselves of endodermal origin" (Boulenger, 1910, p. 767). Thus we have much testimony as to the migration of nematocysts within the tissues of cœlenterata.

It is to be noted, however, that *in all these cited examples the* *nematocysts are transported while enclosed by cnidoblasts.* In the twenty nematocysts found within the endoderm my material shows the stinging-threads lying free within vacuoles at the bases of the endodermal cells. Martin also describes the nematocysts that he noted within the endoderm of *Microstoma* as being free from nematocytes or cnidoblasts. In these cases, therefore, either the cnidoblasts after having carried the nematocysts into the endoderm have died or the stinging-cells were not taken into the endodermal tissue by cnidoblasts. Judging from what has been seen I am of the opinion that these nematocysts have found their way into the endodermal cells through the selective action of the latter. Such selective faculties have also been observed for the cnidophages of the ceras of æolids: Foreign bodies within the lumen of the cnidophore of æolids have been found "by Heckt, by Hancock and Embleton, and by Grosvenor. I have found uninterpretable fragments of tissue, bodies not tissue-like, and diatoms, in the liver diverticula and in the cnidophores. The ciliated canal has no power to distinguish other indigestible bodies from nematocysts, but this inability to select is not shared by the cnidophages, for so far as I know, these ingest only nematocysts" (Boulenger, 1910, pp. 127–8).

The cnidophages of æolids deliver their nematocysts to the cnidocyst, whereas the endodermal cells of *Microstoma* deliver their nematocysts to the mesoderm.

In the material of this laboratory numerous nematocysts are found within the mesoderm. The nematocysts in the mesoderm that lie nearest the endoderm are in all cases enclosed in a vacuole. These vacuoles are similar to those described and figured by Martin and Vallez. About each vacuole the mesoderm crowds. Two kinds of cells of the mesoderm are to be found in or near the walls of these vacuoles: (*a*) a branched type of cells, which form the frame-work of the mesoderm. These cells have nuclei whose chromatin granules are conspicuous but more or less equal in size. The cytoplasm of this first type of cell is not very dense and is greatly branched, its branches anastomosing with those of its fellows (Fig. 6, A); (*b*) cells which are much less frequent in their occurrence, with denser, more compact cytoplasm than that of the first type of cell, the nuclei of the second kind of cells being characterized by having a relatively large black nucleolus which lies in a vacuole (Fig. 6, *B*). These are taken to be the wandering cells or amoebocytes of *Microstoma*.

When the nematocysts are found lying nearer the ectoderm cytoplasmic strands appear extending more or less into the vacuoles about the nematocysts. At *P*, in Fig. 6, is represented what appears to be pseudopodia of a wandering cell invading a vacuole.

Whether or not in this and similar vacuoles there is evidence of amoebocytes entering the vacuoles, the *vacuoles* in all cases are filled by a cell when the nematocysts come to lie near the ectoderm. About the nematocysts that have but recently reached the ectoderm the cytoplasm and nucleus of the invading cell are much more evident than in the cases where the nematocysts have been at the surface sufficiently long or near to suffer discharge (Figs. 7 and 8). In other words the cells seem to spend their vitality in caring for these exotic nematocysts and about the partially discharged nematocysts are to be found cytoplasm and nuclei which indicate a depleted condition (Figs. 9 and 10).

The nuclei of those cells do not lie at the surface of the vacuole as Martin shows in his figure number four, plate 14, as he describes in his legend for this figure. The nucleus in each case can be seen to lie well within the lumen of the original vacuole. This may be determined by the study of transverse or longitudinal sections. Figs. 7 and 8 represent two contiguous sections of a nematocyst near the ectoderm. Fig. 7 was taken through or near the equator of the nematocyst. Fig. 8 represents the section taken immediately following that shown in Fig. 7. In the eighth figure the lower surface of the section is shown. When the focal plane is raised above this level three microns the nuclei of the overarching mesodermal cells appear and the nucleus of the invading cell disappears. Thus it is determined beyond doubt that there is intimately associated with the exotic nematocysts, when they come to lie near the ectoderm, a cell which has invaded the vacuole, that had appeared about the nematocyst within the endoderm. This cell can be seen in fresh material as described earlier in this paper and in all of my permanent preparations.

The question naturally presents itself as to what kind of cell it is that has thus entered the vacuole. In all favorable nuclei that are found within the vacuole there is present a deeply staining nucleolus about which there is to be seen a vacuole (Figs. 8 and 10). This makes the nuclei more closely resemble those of the amœbocytes than those of the other mesodermal cells of the *Microstoma*. So I conclude that in each case an amœbocyte enters the vacuole to take charge of the nematocyst as do the cnidophages of the æolids.

It is interesting to observe how these nematocysts are handled by the invading cell of each vacuole.[1] The nematocysts when they lie alone within the vacuoles are pointing indifferently in all directions, but after they have been taken charge of by the cnidophages they have in all instances their discharging poles directed towards the exterior of the body. Martin also observed this final exact orientation of the nematocysts. He says of it "the large barbed nematocysts in their final position, always lie so that the thread, when it is discharged, will pass out of the animal, although they may lie pointing in any direction while they are still in the gut cells or the body-cavity. This rule does not seem to hold good in the small cylindrical nematocysts, which, as far as I can see usually lie almost parallel to the surface. It is very difficult to say how such an orientation can be effected, but something of the same kind has been detected in *æolids*, and I believe that the same difficulty is present in the nematocysts of the tentacles in cœlenterates" (Martin, 1908, p. 267). It would indeed be a most difficult matter to understand their orientation if Hallez and Martin are correct in describing these exotic nematocysts as always lying within vacuoles. The cnidophages appear to be the vital agents which properly orient the nematocysts.

In my preparation one barbed nematocyst was observed that had been apparently inaccurately discharged. This was taken from near the middle of a specimen that when fixed remained extended. In such distended specimens the mesoderm near the middle of the animal is reduced to a very thin layer; this together with a possible shifting of the mesoderm at the exact time of discharge may reasonably account for this single exception that is represented in Fig. 11.

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[1] Hereafter we shall speak of this invading cell of the vacuole as a "*cnidophage*."

There is a second type of nematocyst found in *Hydra*. These are the so-called "grappling" nematocysts. By means of them the *Hydra* lays hold of or entwines objects of prey; when the prey is thus seized the tentacles of *Hydra* sweep it into the mouth of the polyp. This seems to be the chief function of these nematocysts; but the poison sac at the base of this type of nematocysts suggests that they are something more than mere prehensile structures. If they are not more than this, I wonder at *Microstoma* taking them into its tissues and passing them out to its surface where they are, so far as my observations are concerned, always oriented as are the barbed nematocysts. Frequent nematocysts of this type have been found by me within sections of *Microstoma*. In some cases these grappling nematocysts lie within vacuoles in which no attending cell or cnidophage can be recognized. Fig. 12 shows such a nematocyst lying near a vacuole from which it may have been discharged. These nematocysts are small and their vacuoles likewise small so that it is difficult to determine whether cells, associated with them, lie within their vacuoles or not; but frequent nematocysts of this type have been found that have attending cnidophages within their vacuoles (Fig. 13). Moreover all the exotic "grappling" nematocysts were so oriented that their discharging poles were directed towards the surface.

I have come to look upon the nematocysts of *Microstoma* as being derived from *Hydra*. This conclusion is based upon the following facts: (1) that there is a great variation in the number of nematocysts of *Microstome*—from very few to many; (2) the close resemblance between the nematocysts of *Hydra* and *Microstoma*; (3) the absence of cnidocils in *Microstoma;* and (4) the absence of cnidoblasts about the nematocysts when found within the endoderm and deeper mesoderm of *Microstoma*.

Toward what end is all this careful handling of the exotic nematocysts of *Microstoma* directed?

Glaser (1909) has determined by experiment that nematocysts will not be discharged in the digestive fluids of æolids.

There would be no protection gained by the flatworm in passing them into the tissue of its body if in the digestive fluids of *Microstoma* the nematocysts are likewise not discharged; and in case they did discharge within the digestive fluids of *Microstoma* they would have done their injury before they had been taken up by the tissues of the *Rhabdocœle*.

Glaser (1909) has further demonstrated that when nematocysts pass from digestive fluids into fresh water in all cases they discharge. Here then are seen grounds for making the inference that his careful handling of the nematocysts is done in order to prevent their haphazard discharge in the region of the mouth as they are egested. Over against this inference is the fact that in æolids certain nematocysts pass from the alimentary canal with the waste food while others are more carefully handled and discharged by the cnidophages of the cerata (Glaser, 1909): also when a flatworm is bold enough to attack a living *Hydra* with its anterior end it can hardly be considered to be so serious a matter to have nematocysts discharged casually within the vicinity of the mouth.

Again the end may be to avoid discharge of nematocysts by means of distortion within the enteron; for Glaser (1909) has shown that nematocysts when distorted do discharge. This suggestion I think can be dismissed with the inference that within the mesoderm the nematocysts are just as liable to distortion as within the enteron; for through the muscular actions of the body the ova are seen at times to suffer considerable distortion.

The third suggestion is that these bodies are taken up to be used. Grosvenor says: " 'No one can have witnessed the reaction of an æolid to various stimuli . . . without being convinced that the cerata are used as a means of defence' " (Glaser, 1910, p. 138). Likewise no one can have witnessed the discharge of nematocysts of *Microstoma* when stimulated by pressure or by acetic acid without looking upon them as organs of defense.

It taxes my faith seriously to believe that endodermal cells select nematocysts for so remote a purpose as that of defending the cell-colony at the ectodermal surface, and yet until further evidence can be obtained, if I must take a stand, I conclude that these exotic nematocysts are handled by *Microstoma* for purposes of defense.

If this conclusion is correct and if the nematocysts of æolids are derived from cœlenterata, the question can be raised, among many other more subtle ones, have the æolids acquired their method of dealing with nematocysts of cœlenterata through flatworm ancestry, or are these cases of parallel development of reflexes or instincts?

I hope to be able to make a series of experiments upon the nematocysts of these *Rhabdocœles*.

Summary.

The nematocysts of *Microstoma* are derived from *Hydra* upon which *Microstoma* feeds. These ingested nematocysts are delivered to the mesoderm by the endoderm of the flatworm. Within the mesoderm an amœboid cell takes charge of each nematocyst and as it is transported to the surface orients it so that the "sting-thread" has its discharging pole directed towards the exterior of the animal. Thus *Microstoma* has come to use the nematocysts of its prey as do the æolids use the nematocysts of their prey.

Biological Laboratory,
University of Virginia,
March, 1911.

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Explanation of Plate I.

Fig. 1. Nematocyst with its associated cell. Obtained by gentle pressure of cover-glass upon a specimen treated with a drop of 1 per cent. acetic acid. Free hand drawing, with aid of micrometer eye-piece. ×1,800.

Fig. 2. Same cell after lying ten minutes in the dilute acetic acid. Vesicle has formed at one end of cell. Free-hand drawing, with aid of micrometer eye-piece. ×1,800.

Fig. 3. Anterior end of *Chætogaster*. Free-hand drawing.

Fig. 4. Setæ taken from lumen of enteron of sectioned *Microstoma*. Camera lucida drawing. ×500.

Fig. 5. Endodermal cell. Nematocyst lying within vacuole, *V*, at base of cell; *M*, basement membrane of endoderm. Camera lucida drawing. ×1,500.

Fig. 6. Nematocyst within vacuole, *V*, lying near middle region of mesoderm; *A*, supporting cell of mesoderm; *B*, wandering cells of mesoderm; *E*, ectoderm; *M*, basement membrane of endoderm; *P*, pseudopodium (?) of wandering cell. Camera lucida drawing. ×1,500.

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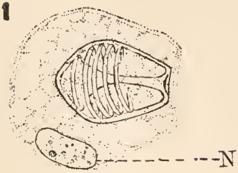

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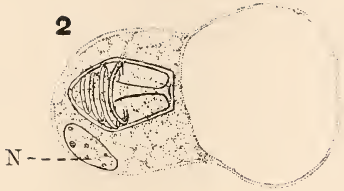

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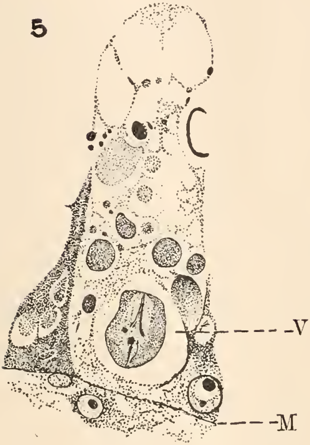

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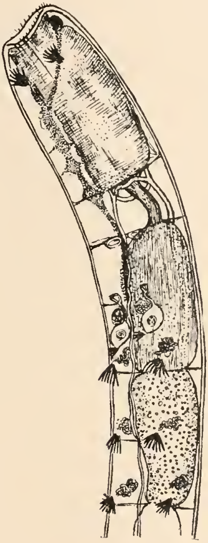

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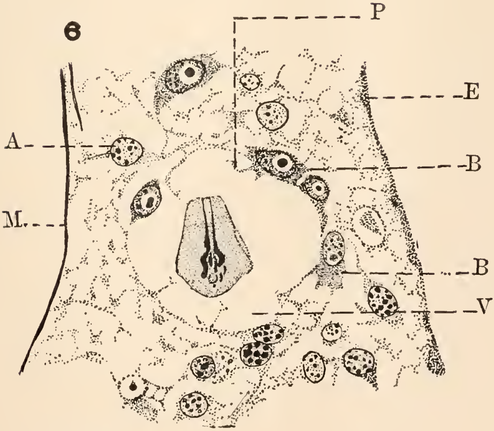

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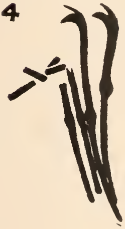

W. A. KEPNER

EXPLANATION OF PLATE II.

FIG. 7. Undischarged nematocyst with its vacuole filled by cytoplasm. It lies near the ectoderm, *E*. Camera lucida drawing. ×1,500.

FIG. 8. Section next to that represented in FIG. 7. Shows the nucleus, *N*, of cell associated with the nematocyst. Camera lucida drawing. ×1,500.

FIG. 9. Partially discharged nematocyst. *E*, ectoderm; *M*, basement membrane of endoderm. Camera lucida drawing. ×1,500.

FIG. 10. Partially discharged nematocyst. *N*, nucleus of cell associated with nematocyst. Camera lucida drawing. ×1,500.

FIG. 11. Nematocyst discharged approximately parallel to the surface. *E*, ectoderm; *M*, basement membrane of endoderm. Camera lucida drawing. ×1,500.

FIG. 12. Grappling nematocyst, partially discharged. *E*, ectoderm, *M*, basement membrane of endoderm. Camera lucida drawing. ×1,500.

FIG. 13. Grappling nematocyst, partially discharged. An attending cell or cnidophage is shown within the vacuole within which the nematocyst lies. Camera lucida drawing ×1,500.

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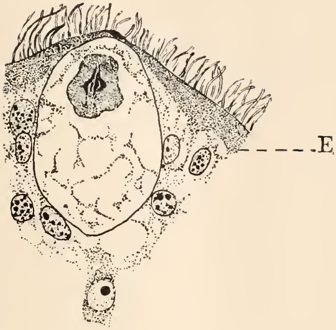

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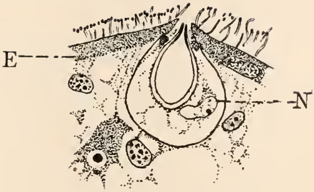

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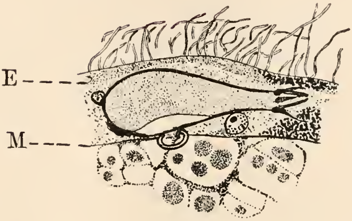

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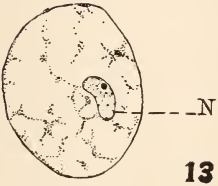

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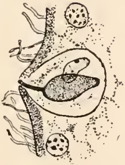

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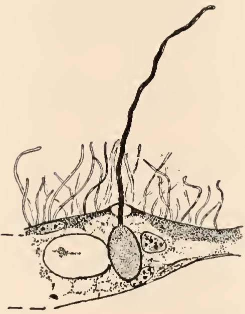

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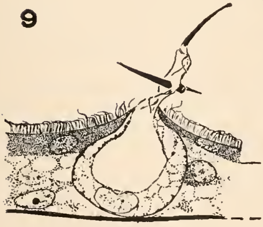

EXPERIMENTS ON THE ADOPTION OF LASIUS, FORMICA AND POLYERGUS QUEENS BY COLONIES OF ALIEN SPECIES.[1]

MAURICE COLE TANQUARY.

The experiments recorded in this paper were performed during the past summer in the laboratory of the Bussey Institution, Harvard University, at the suggestion and under the direction of Professor W. M. Wheeler. At the beginning of this particular series of experiments I had in mind only two species with which I intended to work, namely, *Aphænogaster tennesseensis* and *Aphænogaster fulva* with its subspecies *aquia* and variety *picea*, but a number of field excursions taken during July and August brought to hand several other species which served as such excellent material for similar experiments that it was decided to include them also.

Object of Experiments.

The object of these experiments was to determine whether the queens of certain species of ants are parasitic upon certain other species in founding their colonies. The various methods employed by queen ants in founding their colonies are stated in Wheeler's "The Founding of Colonies by Queen Ants," and since the following experiments have a direct bearing upon that question, and in fact form a continuation of exactly the same sort of work, I think I can do no better in the beginning than quote a few paragraphs from that paper.

"Female, or queen, ants in founding their colonies resort to one of three methods, which may be known as the usual or typical, the redundant, and the defective. . . .

1. "The female ant is able by herself alone to start her colony; that is, under favorable circumstances she can produce and bring to maturity the first brood of workers and thus insure the further growth and development of the colony. She is capable of passing many months without nourishment even while she is feeding her off-spring. Her voluminous fat-body, built up during her larval life in the maternal nest, together with her degenerating wing-muscles, furnish the sub-

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[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 29.

stances that are converted into food for the young. Although so arduous that few of the many queens of all that celebrate their nuptial flight during a season ever succeed in founding a colony, this method is, nevertheless, the one adopted by the great majority of ants."

The second method refers to the fungus-raising ants (Attii) and need not be given here.

"3. The female ant, owing to her small and delicate stature, or delayed fertility, is quite unable to found a colony without the aid of workers of another species. This method, which is resorted to by parasitic species—using that term in a very broad sense—appears under three different aspects.

"A. As temporary social parasitism. The female seeks and obtains adoption in a small queenless colony of another species and permits its alien workers to bring up her young. When these have matured, they emancipate themselves and become an independent colony, either by emigration or, more probably, only through the natural death of the host species."

"B. As permanent social parasitism. The female seeks and obtains adoption in a colony of some other species and there permanently resides together with her offspring. Examples: *Anergates*, *Strongylognathus*, *Protomognathus*, *Wheeleria*, etc.

"C. As dulosus, or slavery. The solitary female enters a small colony of another species, kills the workers, and seizes and rears the progeny (larvæ and pupæ) as a first step towards bringing up her young. The workers produced by the female subsequently make forays on other colonies of the host species and appropriate their offspring. While they use a portion of these as food they permit another portion to develop as 'auxiliaries' or 'slaves' so that the colony preserves its 'mixed' character."

In the same paper and in others Wheeler has also brought out the fact that parasitic species are sporadic in their occurrence, that they usually produce a very large number of females which are aberrant in some way, such as being of unusually small size, sometimes smaller than the workers, in being mimetic in coloration or behavior, in being covered with fulvous, myrmecophilous hairs, etc. Therefore the possession of the above characteristics to any extent by a species of ant is good grounds for believing that the queens of that species may be parasitic in founding their colonies.

A paragraph on page 447 of Wheeler's book, "Ants, their Structure, Development and Behavior," gives the reasons for performing adoption experiments with queens of *Aphænogaster tennesseensis*.

"The female of the myrmecine ant *Aphænogaster tennesseensis* in being deep red and of very small size, with a glabrous body and huge, flattened, epinotal spines protecting the vulnerable abdominal pedicel, is so unlike the females of any other members of the genus *Aphænogaster* that she was originally described by Mayr as the type of a distinct species (*A. levis*). These peculiarities suggest temporary parasitism and this is borne out by the observations of Schmitt and myself (1901*c*). Schmitt found near Beatty, Pa., a small mixed colony of *A. tennesseensis* and *A. picea*, a variety of *fulva*, and one of the commonest ants in the northern states. He was impressed with the fact that the nest of this colony was under a stone, because *tennesseensis* normally nests only in rotting wood. During the summer of 1902 I found near Rockford, Ill., two mixed colonies like that observed by Schmitt, except that the variety *picea* was represented by the variety *rudis*. Both colonies were of small size and situated under stones. In one of them a *tennesseensis* queen was unearthed. There can be little doubt, therefore, that the glabrous queens seek out small nests of some variety of *fulva* and start their colonies in them just as *consocians* does in the nest of *incerta*. This habit is also indicated by the sporadic distribution of *tennesseensis* and its occurrence only in localities where some form of *fulva* is abundant."

The reasons for performing adoption experiments with queens of the other species will be given as I take up those experiments.

METHOD.

The method used in these experiments consisted in placing artificially dealated queens of the supposedly parasitic species in a nest with a few workers and brood of the supposed host species and observing their behavior under these conditions. This is the same method used by Wheeler in the experiments recorded in the paper mentioned above, and possible objections to it are answered by stating that an artificially dealated queen behaves in the same manner as a fertilized queen that has dealated herself, and that if one is to conduct such experiments on a large scale it is necessary to use artificial nests and to prevent the escape of the introduced queen. For most of the experiments I used large Petri dishes 4 1/2 and 6 in. in diameter into which I dumped the ants with their brood and some earth from the nest. For some of the experiments, however, I used nests of the Fielde pattern, and in that case I always introduced the queen into the light chamber and allowed her to find her own way into the dark chamber where most of the ants stayed, thus imitating natural conditions a little more closely. When I used Fielde nests, one chamber was kept covered with a piece of orange-colored glass. A few of the experiments with each species I watched very closely, especially when the queen was first introduced, sometimes watching a nest almost constantly for several hours, but as I had a large number of experiments going at the same time, sometimes more than fifty, I had to be content with examining most of them hastily several times a day and depending chiefly upon the final result which, after all, is the important thing.

APHÆNOGASTER TENNESSEENSIS.

As this species does not occur in eastern Massachusetts I collected part of a large colony from a wood near Urbana, Ill., June 8 and brought it with me a few days later to the Bussey Institution, where I transferred the ants to a plaster of Paris nest, made on the Fielde pattern. The nest contained only workers and a large number of larvae. By the middle of July winged females began to appear and during the summer more than a hundred were produced. These, and a few sent me from Illinois later in the summer by Messrs. Hugh Glasgow and R. D. Glasgow, were the ones used in the experiments. Altogether I tried thirty-five queens of *A. tennesseensis* with eleven different colonies, two of which were *A. fulva* subspecies *aquia* var. *picea*, one *A. fulva*, and the others *A. fulva aquia*. The latter is probably more common where *A. tennesseensis* occurs. Of these thirty-five queens I got but one clear case of adoption and unfortunately this was with one of the colonies for which I have but scanty notes. The notes of this experiment are as follows:

EXPERIMENT B. 24*c*.

Aug. 18.—3.00 P.M. I place an artificially dealated queen of *Aphænogaster tennesseensis* in a Petri dish with five workers and about fifty pupæ of *A. fulva aquia*.

Aug. 19—9.00 A.M. Queen standing by herself.

Aug. 20—9.30 A.M. The same.

Aug. 21—11.30 A.M. She is standing on the pile of pupæ with the workers, none attacking her.

Aug. 22. The same.

Aug. 23—8.30 A.M. She stays in the midst of the workers and pupæ and seems to have been adopted.

Aug. 23—3.30. Still with the workers—they never attack her.

Aug. 24—8.00 A.M. In the center of the bunch of workers; about three dozen of the pupæ have become callows.

Aug. 28. Still being treated as their own queen.

Sept. 10. I have examined her every day up to the present time, and have never seen her treated by the workers other than as their own queen. There are now about sixty workers.

In all the experiments with *Aphænogaster tennesseensis* the queen was attacked by the workers, usually at the very outset, sometimes not until she had been in the nest for a few minutes.

Her behavior was about the same in most cases; she was timid and either ran away or crouched down in a supplicatory posture. Some of the queens, however, showed no fear whatever at first and approached the *fulva* workers as though they were members of the same colony. Constant biting and pulling about by the legs and antennæ, however, soon caused them to try to escape from their tormentors. In nearly every instance the behavior of the introduced queen was conciliatory and in no case did she at first seem to resent her harsh treatment but seemed to try either to conciliate the *fulva* workers or to escape from them. One queen, however, as will be shown in the following experiments, departed widely from what is apparently the normal behavior under such conditions by seizing a worker and carrying it about when the others attacked her. At first this behavior seemed to render her almost immune from further attacks and I began to think that perhaps only such queens as showed this particular phase of behavior succeeded in getting themselves adopted. Later on, however, the attacks were renewed and the queen was finally killed. I will give one experiment in detail as a type, and summarize the others.

EXPERIMENT B. 18.

Aug. 9—9.00 A.M. I place an artificially dealated queen of *Aphænogaster tennesseensis* in the light chamber of a Fielde nest containing about two dozen workers and a small pile of larvæ and cocoons of *A. fulva aquia*. She goes at once into the dark chamber. The first few workers that she meets recognize her at once as a stranger, but do not attack her. Several threaten her and then pass on. After about five minutes one worker grabs her by the petiole and carries her about in the nest. She does not seem afraid and does not try to get away; her manner is conciliatory.

9.10 A.M. She goes out into the light chamber but soon returns.

9.20 A.M. One worker is tugging at her antennæ.

9.25 A.M. The same worker is still holding her.

9.27 A.M. She gets free from the worker and runs around in the dark chamber. Many of the ants she meets do not attempt to attack her.

9.30 A.M. Another worker catches her by an antenna and holds her for a while.

9.44 A.M. Two workers lick her for about two minutes and then go away.

9.47 A.M. A worker seizes her by one of her tarsi and pulls her about for a while.

9.48 A.M. Three workers are licking her. She is resting on the sponge about one half an inch from the brood. A number of workers keep passing by her; some of them stop and lick her a while, some feel her over with their antennæ, open their mandibles as though to attack her and then go away without doing so. Sometimes she pats the heads of the workers with her antennæ as though begging food.

10.09 A.M. One worker catches her by a middle leg and pulls her about for a minute, then stops and licks her thorax and abdomen, then seizes her again by the leg and pulls her, but apparently not very hard. Again it stops and licks the queen for a minute and then goes a little to one side and begins cleaning its own antennæ but soon comes back to the queen and repeats the whole performance. The worker keeps this up for twelve minutes and then goes away. The queen has remained all this time in the same position, one half inch from the brood.

2.00 P.M. She is out in the light chamber but soon goes back.

4.35 P.M. One ant holding her by the petiole, another by one antenna.

6.00 P.M. A worker holding her by the antennæ.

8.15 P.M. The same.

Aug. 10—7.15 A.M. She is out in the light chamber alone.

9.15 A.M. She is in the dark chamber but not with the workers.

11.30 A.M. She has been in the light chamber by herself most of the morning.

11.50 A.M. Two workers are pulling her about.

2.00 P.M. She is running around in the light chamber, trying to get out. The workers seem more hostile today than yesterday.

Aug. 11—8.00 A.M. The queen is dead; I remove her.

9.05 A.M. I place another queen in the light chamber. She starts into the dark chamber and is seized in the passage way by two of the workers, one pulling an antenna, another holding her petiole. They drag her about in the dark chamber.

9.50 A.M. The queen is on the sponge near the brood and has seized the petiole of one of the workers in her mandibles. Workers are standing about but do not attack her; once in a while a worker licks her.

10.30 A.M. A worker seizes her by the hind tarsus and holds her for five minutes. She is still holding the worker.

10.15 A.M. Still in the same place. She is straddling the head and thorax of the worker which she is holding by the petiole. Once in a while a worker seizes her by a leg or an antenna, but some of the workers are licking her.

10.30 A.M. Still holding the worker.

11.10 A.M. The same.

12.10 P.M. The same.

1.30 P.M. The same. She is standing in the midst of the brood.

3.50 P.M. Still holding the worker; she is carrying it around now on the sponge and in the midst of the other workers and brood. She pays no attention to the other workers, some of which nab her at times. There is no excitement in the nest.

4.30 P.M. Still carrying the worker around; she does not get far from the sponge and most of the time is on it with the brood and workers. The worker seems to be nearly dead and for several hours has made no effort to escape or defend itself.

5.00 P.M. The same.

6.00 P.M. The same. A worker holding the queen by the antennæ.

8.00 P.M. The queen is still on the sponge holding the worker.

9.00 P.M. She is about two inches from the sponge, a worker holding her by the antennæ; she is not holding the worker now.

Aug. 12—8.00 A.M. She is again on the sponge with the brood, a worker tugging at one of her tarsi.

9.00 A.M. She is about two inches from the sponge, not holding the worker, but one of them is holding her by the antennæ.

9.15 A.M. She is again on the sponge with the brood and is holding the worker by a middle leg. The worker seems to be helpless in her grasp and does not attempt to escape.

9.22 A.M. I change the orange glass in order to make the other chamber the dark one.

9.35 A.M. The brood has all been removed to the moist sponge in the other chamber. A few ants still running about in this (now light) chamber. The queen and her victim still on the sponge. The other ants sometimes come up and feel them over with their antennæ but do not attack the queen.

10.00 A.M. During the past forty-five minutes the queen has been standing almost motionless. She now begins to move about on the sponge, probably because all the other ants are gone. At times she stands on her middle and hind feet and with her front feet seems to try to twist or change the position of the worker she is holding. She bends under her abdomen as though spraying the worker with formic acid.

10.10 A.M. She leaves the sponge and carries the worker about for a minute and then comes back.

11.30 A.M. She has remained on the sponge all this time holding the worker.

11.40 A.M. She is alone on the sponge. I do not know whether the worker escaped or whether it died and she dropped it. There are several dead workers in this chamber.

11.45 A.M. She is running about in the light chamber and seems to be entirely uninjured.

11.50 A.M. She goes into the dark chamber, seizes a worker by the thorax near the petiole (almost exactly the same hold she had on the first worker) climbs on to the sponge and stops just in the middle of the brood and workers some of which pull her legs or antennæ, but she retains her hold on the one worker and remains in the same position. I remove all the dead workers from the nest.

1.40 P.M. Still holding the worker in the same way.

3.15 P.M. The queen is still holding the worker and is staying with the brood.

6.15 P.M. The same.

Aug. 13—9.15 A.M. The same. There are no dead workers in the nest so she has not killed any of them.

11.00 A.M. The same.

12.00 M. The same.

1.25 P.M. The queen is not holding the worker but three of the workers are holding her, one by the petiole, one by an antenna and one by a mouth part.

3.00 P.M. Three workers are holding the queen.

6.00 P.M. The queen is again holding a worker and three workers are holding her.

Aug. 14—9.30 A.M. The queen is being stretched out by two workers that are pulling in opposite directions on her legs. She is not holding the worker now.

10.30 A.M. The queen is running about in the light chamber.

6.00 P.M. She is alone in the light chamber, and has two legs and one antenna partly gone.

Aug. 15—9.00 A.M. In the light chamber alone; she has four legs and both antennæ partly gone.

1.50 P.M. The same.

3.50 P.M. The queen is dead and dismembered.

Aug. 16—8.15 A.M. I place in another queen. She runs about in the nest avoiding at first the workers who threaten her. After about four minutes when she is resting on the sponge (not with the brood), a worker comes up and begins licking her at once and continues doing so for more than five minutes.

8.45 AM. The queen is in the corner of the nest feigning death. I thought she was dead at first; several workers standing around her.

9.00 A.M. She gets up and goes over to the brood; after a few minutes a worker drags her away.

9.30 A.M. Three workers holding her.

12.00 M. They are still attacking her.

3.00 P.M. She is being stretched out by four workers.

6.00 P.M. The same.

Aug. 17—7.30 A.M. Three workers are holding her. One leg partly gone.

5.30 P.M. The queen is dead and dismembered.

On August 19 I placed in another queen. She received the same treatment and was killed by August 24. On August 25 I placed in another. This one fared a little better and lived until September 8.

This colony, it will be seen, killed in succession five queens of *Aphænogaster tennesseensis* between August 6 and September 8. That there is a tendency toward adoption is seen in the fact that a number of the workers often licked the queen just as they would their own, while others were attacking her. It seems as though there is something about the queen that is attractive to the workers while at the same time the odor of a different species antagonizes them. In nature the specific odor is probably not so distinct by the time the *tennesseensis* queen enters a *fulva* nest since she will have been out of the parent nest and running about on the ground for some little time. In this and in other adoption experiments only a few ants attacked a queen at once, usually not more than two or three, and very seldom more than five or six, even when the colony was a very large one. It would seem as though the other ants realized that the intruder was being taken care of.

The variety *picea* seemed to be more hostile to the *tennesseensis* queen than *aquia*, but even here a tendency toward adoption is shown, as may be seen from the treatment accorded one of the queens in an experiment from which I take a few notes. This colony contained seventeen workers and a small pile of larvæ of *Aphænogaster picea* and were in a small two-chambered Santschi nest made of glass and plaster of Paris.

July 25—2.45 P.M. I place in the first queen. She receives very harsh treatment and is killed by 4.00 P.M. and her body cut in two at the petiole.

4.10 P.M. I place in a winged queen. She receives no better treatment than her predecessor and is killed by 6.00 P.M.

July 27—9.15 A.M. I place in the light chamber another artificially dealated queen of *Aphænogaster tennesseensis.* She runs about in the light chamber for several minutes, goes into the passage-way several times but not into the dark chamber. The *fulva* workers evidently detect her by her odor and one or two enter the light chamber and dart at her with abdomen turned under as though to grab her, but simply feel of her with their antennæ and allow her to run away.

9.45 A.M. Still in the light chamber.

4.45 P.M. I was compelled to be away during the day. The queen is now resting on the brood and is not being molested by the workers. One worker is licking her, although at times it seems to nab her.

5.30 P.M. The queen is licking the *fulva* larvæ. She moves about among the workers, none of which now molest her in the least. The pieces of the other two queens have been distributed among the larvæ and are being eaten by them.

July 27—8.00 P.M. The queen is resting on the larvæ with the workers and appears to be perfectly at home.

July 28—7.45 A.M. The queen is resting contentedly with the workers and larvæ. I was compelled to leave the experiment for a few days but during my absence one of the students in the laboratory recorded that on July 28—3.00 P.M. hostilities were again begun against the queen and that she was killed by 9.00 A.M. July 31.

Between August 2 and August 26 I placed in the nest in succession seven more queens of *Aphænogaster tennesseensis*, all of which were killed after a longer or shorter time.

The other colony of *Aphænogaster picea* which I used contained about forty workers, one queen, and a number of larvæ and pupæ. I placed the first queen in the nest on July 19 at 2.10 P.M. She was killed some time during the following night.

July 20—8.55 A.M. I place in another queen. She lives until 10.45 the next day. At 10.50 A.M. I place in a third queen and she succeeds in living until the morning of July 23. I also tried two queens of *Aphænogaster tennesseensis* with about twenty workers and some brood of the true *A. fulva.* Both queens were killed. The other experiments with colonies of *A. aquia* may be briefly summarized as follows:

B. 24*a.*

Contained ten workers, two winged queens, one male and brood. Between August 18 and September 1, I placed in three queens all of which were killed.

B. 24*b.*

Contained eight workers and a few pupæ. Between August 18 and September 7 two queens were killed.

B. 24*d*.

Contained eight workers and a number of pupæ. I placed in a queen August 29, which lived until September 4.

B. 24*e*.

Contained seven workers and a number of pupæ. I placed in a queen August 30 and it lived until September 4.

One other experiment with a queen of *Aphænogaster tennessensis* consisted in placing a dealated queen in a Petri dish with about thirty pupæ of *A. aquia*. The pupæ of this species, as with other species of myrmicine ants, are naked and do not require the assistance of workers in hatching. They were collected and placed in a Petri dish with the queen July 23. The queen paid no attention to them whatever. In a few days the first callows began to hatch and busied themselves taking care of the remaining pupæ. The queen at first paid no more attention to the callows than she had to the pupæ and in fact stayed in another part of the nest most of the time. After about a half a dozen had hatched, however, she began to stay with them more of the time. The callows were not hostile to her but readily adopted her as their queen. This, however, is what was to be expected because of the discovery made by Miss Fielde and recorded in her paper "Artificial Mixed Nests of Ants." She says: "If one or more individuals of each species that is to be represented in the future mixed nest be sequestered within twelve hours after hatching and each ant so sequestered touch all the others with its antennæ during the ensuing days, these ants will live amicably together thereafter although they be of different colonies, varieties, species, genera or subfamilies." At the present time, September 10, all the pupæ have hatched and have become adult workers, about thirty in all, and are clustered about the *tennesseensis* queen exactly as though she were their own.

Formica obscuriventris.

This subspecies of *rufa* occurs throughout the northern states east of the Mississippi River. It is fairly common in eastern Massachusetts and has been taken in Illinois. The queens are large, about the size of those of *F. sanguinea* var. *rubicunda*, and have shiny, jet-black abdomens with very little, almost no pubescence. The thorax and head are a darker red than in *F. sanguinea rubicunda*. The workers vary greatly in size.

There are no published accounts of the occurrence of this species in mixed nests in this country, but Professor Wheeler found such a nest this last spring near Boston, and has kindly given me permission to use the note which he made of it at the time. "April 10, 1910. On the northern slope of Great Blue Hill, under a stone, I found a mixed colony consisting of a dealated queen and about 80 workers of *Formica obscuriventris* with about 100 workers of *F. subsericea*. There were a few larvæ evidently of the former species in the nest."

On a trip to that same locality with Professor Wheeler, August 17 we came across a very large colony of this species which extended under several good sized stones from which we secured 10 dealated or partially dealated queens. The fact that they had been partially dealated indicates that they had probably been fertilized and readopted by the colony. The particular slope upon which this nest was found was very rich in nests of *F. subsericea*. I brought the queens of *F. obscuriventris* to the laboratory and also a large number of workers and brood from a colony of *F. subsericea*.

I divided the *subsericea* colony into five groups of workers and brood and tried one or more of the *obscuriventris* queens with each group. The behavior on the part of the queen and workers was the same in each case. Some of the workers attacked the queen, some began at once to lick her and others alternated between licking and biting her. The queen always seemed to show surprise at receiving such harsh treatment so different from what she was accustomed to receiving from the workers in her own nest. Then she would try to get away and finding that impossible would turn and bite a worker, sometimes producing a wound that caused death. If the *subserica* colony was a large one the queen was soon killed, but in the smaller colonies the workers, after a time, ceased their attacks and began to tolerate her presence in the nest, while more and more of them began to treat her as their own queen. I will give full notes on one experiment which shows the difference in results attained in using large and small colonies.

B. *22b.*

August 18—11.30 A.M. I place a queen (I think the mother queen from the colony) of *Formica obscuriventris* in light chamber of a Fielde nest containing about 75 workers, one male and many pupae of *F. subsericea*. These *subsericea* are all very large individuals.

The workers attack her fiercely when she is in the passageway. Some pull her toward the dark chamber while some pull the other way. They finally get her into the dark chamber where six or seven attack her at once.

12.00 M. She is in the dark chamber, workers still attacking her.

1.30 P.M. She is on the sponge in the dark chamber and is motionless. One worker is holding her by a middle leg, three others are licking her.

1.35 P.M. The one worker has let go her hold and four others are licking her. She seems to be dead.

2.30 P.M. The queen is dead. I change the light so as to make the other chamber the dark one.

4.00 P.M. They have moved the dead queen and the pupæ into the other chamber. I change the light again.

4.30 P.M. They again move the dead queen. I change the light again and when about two thirds of the workers are in the other chamber I block the passageway, leaving about two dozen workers, the dead queen and most of the pupæ on one side. I then place in another *obscuriventris* queen with these. They attack her also.

4.45 P.M. Two workers are holding her by the legs and two are licking her.

6.00 P.M. Four workers are holding her by the legs.

August 19—8.20 A.M. Two workers holding her, one by a leg and one by an antenna.

11.45 A.M. Two workers holding her.

2.15 P.M. The two workers still holding her.

3.00 P.M. The queen is dead. I remove the plug and let all but twelve of the workers escape into the other chamber and then replace the plug.

3.50 P.M. I place in another queen with the twelve workers; they attack her. She does not attempt to bite them except when a worker gives her an unusually painful jerk or bite.

4.00 P.M. One worker is licking her and one holding her.

August 20—8.00 A.M. One worker holding her by the leg.

2.00 P.M. Three workers standing near her; none holding her.

6.00 P.M. Two workers holding her.

August 21—11.00 A.M. The queen is standing alone.

August 22—11.00 A.M. She is standing with three workers; they do not attack her.

5.30 P.M. Two workers holding her.

August 23—9.00 A.M. Not attacking her this morning; two workers staying with her.

2.00 P.M. Still standing with two of the workers.

Sept. 10. I have examined this nest several times every day since August 23 and have never seen the queen attacked. There are now ten workers and a number of larvæ and pupæ in the nest, and the queen stays with them all the time exactly as though she were their own.

The other experiments with queens of *F. obscuriventris* may be summarized as follows:

B. *22a.*

August 18. I placed an *obscuriventris* queen in a Petri dish with eight workers and some pupæ of *F. subsericea.* The workers at first attacked her and continued doing so at intervals until noon the next day. After that they did not attack her, but tolerated her presence in the nest and she remained standing by herself until noon August 23. After that she stayed with the workers all the time and was treated as their own queen.

B. *22c.*

August 19. I placed an *obscuriventris* queen in a Petri dish with eight workers and twelve pupæ of *F. subsericea.* She was attacked at first just as the others were and the workers remained hostile until about the middle of the following day when they became indifferent to her presence and by August 25 they adopted her and treated her as their own queen. She had received an injury however in the first attacks from which she did not recover and by the morning of August 27 was dead. There were then six workers in the nest and one of them seemed weak. I removed the dead queen and placed in another. I did not see her attacked after the first day and by a day or two later she was fully adopted by the five workers, the other one having died.

B. *22d.*

August 24. I placed an *obscuriventris* queen in a nest containing about fifty workers, one male and many naked pupæ of *F. subsericea.* She was vigorously attacked, but within five minutes after she was placed in, while four workers were holding her, two others were diligently licking her thorax and abdomen. There were too many hostile workers in the nest however and by the following morning she had been killed and beheaded.

B. *22e.*

August 29—4.00 P.M. I placed an *obscuriventris* queen in a Petri dish with ten workers and eight pupæ of *F. subsericea.* She was attacked by the workers that afternoon, but on the following morning was standing by herself on the opposite side of the dish from where the *subsericea* were with their pupæ, and remained thus for two days. On the third day I disturbed the nest a little, and she moved over and mingled with the workers. They did not attack her and from then on, she stayed with them most of the time and is now, September 10, fully adopted.

It will be seen in the above experiments that of the 8 queens of *F. obscuriventris* tried with workers of *F. subsericea,* 5 were adopted, that these 5 were with colonies of from 8 to 12 workers and that those that were not adopted were with colonies of from 25 to 75 workers. The ease with which these queens are adopted in small colonies of *subsericea,* the decided tendency on the part of many of the workers, even in the large colonies, to begin licking and caressing the queens almost from the first, the inquilinous tendency shown in the behavior of the queens themselves and especially the finding of the mixed colony mentioned in the note above, give conclusive evidence of the fact that the queens of *F. obscuriventris* are, at least in part, temporary parasites on *F. subsericea*, and the fact that a number of partly dealated and therefore probably fertilized queens had been retained in the large colony may be taken as an indication that, once formed, the colony may grow by budding as is the case with the large mound ant, *F. exsectoides*, which has also been shown (Wheeler, 1906) to be temporarily parasitic upon *F. subsericea*.

Lasius (Acanthomyops) latipes.

This species not only possesses aberrant females but is peculiar in the fact that it has two distinct forms of females as shown in a paper by Wheeler and McClendon, "Dimorphic Queens in an American Ant." These two queens have been designated by them as alpha and beta, the beta being the more aberrant but the more common, and up to the time of the appearance of the above mentioned paper, the only one known. The alpha queen, as shown by measurements given in that paper, is almost exactly intermediate between the beta female and the normal female of *L. claviger*. The two more probable of the four possible hypotheses suggested therein in explanation of the occurrence of these two forms are

1. That the dimorphism may be regarded as the result of hybridism between *L. claviger* and *L. latipes*.

2. That it may be a case of true dimorphism in the female sex.

In a recent paper, "An Aberrant Lasius from Japan"[1] Wheeler, after stating that he had made many observations in the field which showed that the queens of the following species of *Lasius*, *L. americanus*, *L. neoniger*, *L. nearcticus*, *L. brevicornis*, *L.* (*Acanthomyops*) *claviger* and *L.* (*Acanthomyops*) *interjectus* are able to establish their colonies independently, adds: "But I have never seen any of the females of our *umbratus* forms (*mixtus* var. *aphidicola* Walsh, *subumbratus* Viereck, *minutus* Emery and *speculiventris* Emery) in the act of founding their colonies independently, and it is quite probable that they are temporary parasites on the extremely common *L. americanus*. Equally negative have been my observations upon *L.* (*A.*) *latpies* which has the alpha and beta females. . . . That this species is a temporary parasite on *L. americanus* is indicated by the fact that near Colebrook, Conn., I found four small mixed colonies." The four mixed colonies mentioned here are the only ones of which there is any published account, but Professor Wheeler found another such colony this last spring and has given me the following note which he made at the time.

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[1] Biological Bulletin, 1910.

"Ellisville, Mass., April 21, 1910 Found a large mixed colony of *L. americanus* and *Acanthomyops latipes*, both about equally numerous and the *latipes* were no larger than the *americanus*. The nest was under a stone and contained a number of larvæ so young that I could not tell to which species they belonged. Both species took part in carrying the larvæ to a place of safety when the stone was raised, but the *latipes* were much more active in this pursuit than the *americanus*. The members of the two colonies were on the most friendly terms and occupied the same burrows."

The above facts furnished very good reasons for trying *latipes* queens with other species of *Lasius* and especially with *L. americanus*. On a field trip with Professor Wheeler in the Litchfield Hills near Colebrook, Conn., we came across a very large colony of *L. latipes* under a stone from which I obtained more than 75 winged females, all but 3 of which were beta females. These, and a number collected about two weeks later by Professor Wheeler, part of them from a colony in the same locality and part of them found crawling over the ground near Canton, Conn., after they had descended from their nuptial flight, were the ones used in the experiment.

Altogether I tried 79 queens of *L. latipes* with 28 different colonies of 8 different species of *Lasius* divided as follows: 14 colonies of *L. americanus*, 4 colonies of *L. nearcticus*, 4 colonies of *L. claviger*, 1 colony of *L. claviger* var. *subglaber*, 1 colony of *L. brevicornis*, 2 colonies of *L. interjectus*, 1 colony of *L. umbratus* var. *minutus* and 1 other colony of *L. latipes*. Out of all these I got but two clear cases of adoption in which the queen lived, one of these being an alpha, the other a beta female. However, not nearly all the deaths among the queens experimented with were due to the hostilities of the other ants, for the queens of this species do not keep well in confinement and during the time the experiment was running more than 100 females not used in experiments died, part of them in the colony with their own workers and part of them in a nest by themselves. I am quite sure that at least four or five and probably more of the queens with *L. americanus* were already adopted or would have been adopted had they not died. In the majority of cases when I removed the dead queen I was unable to find any trace of injury whatever although in a number of cases the body was dismembered and sometimes eaten.

The most noticeable feature about the behavior of the *latipes* female when placed in with a small colony of workers was her desire to be with the brood, although in only one case out of all 79 did I see a queen pick up a larva or cocoon. This queen was in a Petri dish with a few workers of *L. nearcticus* and once when I uncovered the dish she picked up a cocoon and carried it around in her mandibles for about a minute. The queen was always attacked, and in the larger colonies of *americanus* and in the one of *L. umbratus minutus*, very fiercely. I did not see her in any case attempt to defend herself. In the larger colonies she only tried to get away and in the smaller colonies she usually got on the pile of cocoons, while the little workers attacked her and crawled over her large body in their efforts to kill her. Time and again when they succeeded in dragging her away she would return and mount the pile of cocoons. The callows hatching under these conditions would not, of course, be hostile to her and as at this time of the year the callows were emerging in large numbers there would soon be many workers about her that would accept her as their own queen. Even in the larger colonies a number of workers could often be seen licking the queen while others were attacking her.. I will give a few notes on the colonies in which the adopted queens lived, and summarize the others.

B. 19/8 August 9. I place a beta queen of *L. latipes* in Petri dish with twelve workers of *L. americanus* and 150 cocoons.

Aug. 10. The queen is dead.

Aug. 11. I place in another.

Aug. 12. She is on the pile of cocoons with a large number of newly hatched workers.

Aug. 13. Still resting on the cocoons with the callows.

Aug. 14. The queen and callows are clustered together on the brood.

Aug. 15. The queen is almost hidden from sight by the workers that are clustered about her and over her. They are all resting on the pile of cocoons.

Aug. 16. The same.

Aug. 17. The same. Many of the workers licking her.

Aug. 18. She is entirely hidden by the workers clustered over her. I have examined this nest every day up to the present time, September 12, and the workers have always been clustered about her. This is much more pronounced than is the case even with the rightful queen in a colony of *americanus.* There are now more than 100 workers in the nest.

B. 19/13 Aug. 10. I place a beta queen of *L. latipes* in Petri dish with 30 workers and about the same number of cocoons of *L. interjectus.*

Aug. 11. Dead. I place in another alpha queen.

Aug. 12. She is staying with the workers on the pile of cocoons and seems to be perfectly at home.

Aug. 13—9.00 A.M. She is near the pile of cocoons with a bunch of workers. I watch them for 30 minutes this morning and do not see the workers show the slightest sign of hostility although they are touching her and climbing over her all the time.

6.00 P.M. The workers are licking the queen with every evidence of satisfaction.

I have examined this colony every day up to the present time, September 12, and the behavior on the part of both the queen and the workers has always been the same.

In the following experiments not more than one *L. latipes* queen was in a colony at the same time, and although in the summary I make the statement that the queens were killed it should be remembered that probably the majority of them would have died even if they had been in artificial nests with their own workers since more than 100 died that were not used in the experiment.

B. 9.

Colony of 150 workers and brood of *L. americanus;* 1 queen killed.

B. 16.

60 workers and brood of *L. americanus;* 3 queens killed. The last one stayed in the nest 17 days; I think she had been adopted.

B. 17*b.*

300 workers and much brood of *L. claviger;* 1 queen killed.

B. 17*c.*

25 workers, 3 winged females, 5 males, and cocoons of *L. claviger* var. *subglaber.* 3 queens killed, one of these an alpha queen.

B. 19/1. 20 workers of *L. claviger* and brood; 3 queens killed.

B. 19/2. 20 workers and a few cocoons of *L. americanus;* 3 queens killed.

B. 19/3. 20 workers and a few cocoons of *L. americanus;* 5 queens killed.

B. 19/4. 40 workers and 100 cocoons of *L. americanus;* 7 queens killed.

B. 19/5. 20 workers of *L. americanus* and 200 cocoons; 6 queens killed.

B. 19/6. 20 workers and 200 cocoons of *L. americanus;* 6 queens killed.
B. 19/7. 20 workers and 150 cocoons of *L. americanus;* 6 queens killed.
B. 19/8. 12 workers and 100 cocoons of *L. americanus;* 3 queens killed, 1 an alpha.
B. 19/10. 30 workers, 7 queens, no brood of *L. nearticus;* 6 queens killed.
B. 19/11. About a dozen workers, 30 cocoons, of *L. nearticus;* 2 queens killed.
B. 19/12. About a dozen workers, 1 male and 30 cocoons of *L. nearticus;* 1 queen killed.
B. 19/14. 30 workers, no brood, of *L. claviger;* two queens killed.
B. 19!15. 50 workers, many cocoons of *L. nearticus;* 1 killed.
B. 19/16. 36 workers, many cocoons of *L. brevicornis.* 2 queens killed.
B. 19/17. 8 workers and many cocoons of *L. americanus;* 4 queens killed.
B. 19/18. 4 workers and many cocoons of *L. americanus;* 3 queens killed.
B. 19/19. 6 workers and a few larvæ of *L. americanus;* 2 queens killed.
B. 19/20. 30 workers and many cocoons of *L. americanus;* 2 queens killed.
B. 19/21. 30 workers and no brood of *L. minutus;* 1 queen killed.
B. 19/22. 12 workers and no brood of *L. interjectus;* 1 queen killed.
B. 19/23. 25 workers and brood of *L. latipes;* 1 queen killed.

The fact that more queens died in some of the colonies than others has no significance, for in some of them I did not replace the first queen.

We thus have two positive cases of adoption of queens of *L. latipes* by workers of other species of *Lasius* and the five records given above show that *latipes* occurs in mixed colonies in nature. At first thought it might seem that 2 adoptions out of 79 attempts is entirely too small a percentage upon which to base any conclusions whatever regarding the point of temporary parasitism. Yet considering the fact that nests of *L. latipes* are not found abundantly in nature and that the queens are produced in the colonies in large numbers would we expect a larger number of adoptions? It is very doubtful whether even in *L. americanus* 2.5 per cent. of the queens succeed in founding colonies.

Another possible explanation for the mixed colonies, however may be suggested by an observation which I made on September 5. There had been a nuptial flight of *L. americanus* and *L. latipes* and as I was walking through a park reservation not far from the Bussey Institution between five and six o'clock in the evening, I picked up off the ground 30 dealated females of *L. americanus* and 5 dealated beta females of *L. latipes.* As I had but one box with me I placed the queens of both species together and upon returning to the laboratory a few minutes later, dumped them all together in the same nest. After a few minutes the *americanus* queens were huddled together near a moist sponge I had provided for them but the *latipes* queens, always more restless in confinement, were still running about in the nest. They were continually running over or through the bunch of *americanus* queens and sometimes remained with them for several minutes, yet I never saw the slightest signs of hostility either on the part of the *americanus* or the *latipes* queens. The next morning 3 of the *latipes* queens were dead and two days later the other 2 died, yet I feel quite sure that death was not caused by any hostility on the part of the *americanus* queens. In the same length of time 7 *americanus* queens died, but there have been no more deaths up to the present time. I feel sure that a number of the deaths of the *americanus* queens were due to injuries received when I picked them up off the ground. The fact that the nuptial flight of *latipes* and *americanus* may occur at the same time and that the queens of the two species are not hostile to one another suggests the possibility of a colony being founded in common by queens of the two species. This possibility should be tested by experiment. However, I think temporary parasitism a more plausible explanation of the mixed colonies mentioned above because of the fact that the *latipes* queen is of a more nervous temperment, and even though there were no hostilities between the two queens she would not be satisfied to settle down in a little cell with the phlegmatic *americanus* queen and wait nine or ten months for the appearance of workers. This nervous disposition, however, is exactly suited to running about over the ground until the queen happens to run into a small Lasius colony, and when she gets on to the brood she is perfectly satisfied to settle down as is shown by the experiments.

The adoption of the *latipes* queen by the colony of *L. interjectus* may be looked upon as adding weight to one of the explanations quoted above for the occurrence of the two forms of females, namely, that the alpha form may be a hybrid between the beta female and a male *claviger*. Since adoption occurred with *interjectus* it might also be expected to occur with the nearly related *claviger* if enough cases were tried. Again the females of *claviger* and *interjectus* are very similar so that the alpha form might just as well be a hybrid between *latipes* and *interjectus* as between *latipes* and *claviger*.

LASIUS UMBRATUS VAR. MINUTUS.

One of the quotations given above shows the lack of evidence that the queens of this species are able to found their colonies independently. The lack of such evidence taken together with the fact that the ant is sporadic in its occurrence, that it produces an immense number of the sexual forms and that the females differ from all the other *Lasius* females in being very small, no larger than the largest workers, point to temporary parassitism as a method of colony formation.

On August 12 I came across a large mound nest of this species at the edge of a forest reservation near the Arnold Arboretum. The mound was in the shape of a very broad dome, about eighteen inches high and about three feet across at the base. Judging from the size of the nest, the number of individuals and the way the grass was shot up through the mound the colony must have been several years old. With a trowel I took out about a quart of dirt from the side of the mound and brought it to the laboratory. I found that I had about 150 females, an equal number of males, several hundred workers and many cocoons. As the amount of earth I removed hardly made an impression on the mound the colony must have contained several thousand males and females and a still larger number of workers. Although I collected rather extensively in that region and in a number of other localities around Forest Hills, this is the only colony of *minutus* that I found during the entire summer. In my experiments with this species I used 88 queens in 20 different colonies as follows.

B. 25*a*.

20 workers, 2 winged queens and brood of *L. americanus*.

B. 25*b*.

8 workers and many pupæ of *L. americanus*.

B. 25*c*.

8 workers and many pupae of *L. americanus*.

B. 25*d*.

30 workers and many pupæ of *L. americanus*.

B. 28/1. 7 workers, no brood of *L. claviger*.

B. 28/2. 24 workers and a few cocoons of *L. americanus*.

B. 28/3. 12 workers, and brood of *L. americanus.*
B. 28/4. 6 workers and a few larvæ and pupæ of *L. americanus.*
B. 28/5. 100 workers, and cocoons of *L. americanus.*
B. 28/6. 100 workers, and cocoons of *L. americanus.*
B. 28/7. 75 workers, 1 winged queen and many ocoons of *L. americanus.*
B. 28/8. 12 workers, no brood of *L. interjectus.*
B. 28/9. 25 workers, and brood of *L. americanus.*
B. 28/10. 25 workers and 5 winged queens of *L. nearticus.*
B. 28/11. 24 workers, and cocoons of *L. americanus.*
B. 28/12. 100 workers and many cocoons and young larvæ of *L. americanus.*
B. 28/13. 12 workers, and cocoons of *L. americanus.*
B. 28/14. 24 workers, and cocoons of *L. brevicornis.*
B. 28/15. 50 workers, and cocoons of *L. nearticus.*
B. 28/16. 30 workers and a number of cocoons of *L. americanus.*

The queen of this species is very active and very timid. I did not see her attempt to defend herself in any case but always tried to escape and by her active movements she was usually able to get away from the workers for quite a while. When caught, however, she usually succumbed in a much shorter time than was the case with the other species with which I worked. A few of them died within an hour or so after being placed in, others living for several days. Out of all these experiments I got but one case of adoption. I will give a few notes from that experiment.

B. 25*b*.

Aug. 18—3.30 P.M. I place a queen of *L. umbratus* var. *minutus* in a Petri dish with 8 workers and a large number of pupæ of *L. americanus*. The workers attack her; she does not defend herself but tries to escape from them.
Aug. 19—9.05 A.M. She is running around by herself. A worker attacks her for a little while but she gets away.
Aug. 20—9.30 A.M. She is resting on the cocoons with the workers.
Aug. 21—9.00 A.M. The same.
11.00 A.M. Still on the cocoons with the workers and seems to be entirely immune from attack. About a dozen callows have hatched.
Aug. 22. The same.
Aug. 23. The same.
Aug. 24. There are about 40 or 50 workers in the nest now. The queen stays with them all the time.
Sept. 12. Most of the cocoons have hatched. The queen has been fully adopted.

Although one adoption out of 88 attempts is a small percentage, yet I think the ease with which this queen was adopted is very suggestive, and taken altogether with the facts mentioned above, namely the sporadic occurrence of the species, the very large number of females produced, the small size of the females, the fact that these females have not been seen in the act of founding a colony and one additional fact which may be mentioned, the mimetic coloration of the females (the color of these females is exactly the same as that of the darker form of *americanus*), I think justifies us in concluding that the queen of this species is in all probability, temporarily parasitic upon the common *L. americanus*.

Polyergus lucidus.

This shining slave-maker has been studied by Mrs. Treat, McCook, Burill, and Wheeler,[1] and the European form *P. rufescens*, by Huber, Forel, Wasmann and Viehmeyer. It differs from the ants mentioned above in that it is not a temporary but a permanent parasite or slave-maker, the workers making raids upon colonies of *Formica schaufussi* and its varieties *incerta* and *nitidiventris*.

Studies by Forel, Wasmann and Viehmeyer on the European *P. rufescens* tend to show that that form resembles the temporary parasites in that the queens may be adopted by fusca workers. In regard to the founding of colonies by *P. lucidus* Wheeler says:[2] "Several experiments in which I introduced artificially dealated queens of *lucidus* into nests containing *incerta* workers with their brood gave rather conflicting results. In some cases the *lucidus* queens behaved like the *sanguinea* queens under similar conditions to the extent of killing the alien workers, but they paid absolutely no attention to the brood. In other cases they were passive and conciliatory but equally indifferent to the *incerta* cocoons. It will be necessary therefore to study this question further before making definite statements in regard to the methods employed by our American amazons in establishing colonies."

The queens used in the following experiments were obtained on the slope of Blue Hill, August 17, the same date upon which we found the large colony of *F. obscuriventris* mentioned above. The nest was along the side of a little-used roadway. On excavating and bringing it to the laboratory I found the colony to contain about a dozen workers, an equal number of winged queens, one dealated queen of *P. lucidus*, and about 100 very small workers of *F. incerta* and a number of cocoons. The small number of *lucidus* workers and the comparatively large number of females indicates that many of the workers were probably out on a slavemaking raid at the time.

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[1] See Wheeler, "Ants, Their Structure, Development and Behavior," pp. 482–487.

[2] "Ants, Their Structure, Development and Behavior," p. 486.

I tried seven queens with colonies of *F. incerta* and four with *F. schaufussi*. Two out of the seven were clear cases of adoption in which the queens are still living. One of the others I think was without doubt adopted but died later on from injuries received in the first struggles. The two *schaufussi* colonies consisted of very large individuals and each contained a queen. They attacked the *lucidus* queens more fiercely than did the *incertas*, and all four were finally killed, yet in both colonies I saw at times a few of the workers licking the queens and I think it would be possible if enough cases were tried, to secure adoption with *F. schaufussi*, especially if the colonies did not contain queens of their own.

The behavior of the *lucidus* queens was like that of the *obscuriventris* queens in that they did not at first attack the workers but ran about in the nest avoiding them and trying to escape. However, when caught and held by the workers they were not nearly so submissive as were the *obscuriventris* queens and when forced to defend themselves they did so with such vigor and persistence, pierceing the head or thorax of the unfortunate workers with their long sickle-shaped mandibles so that a very large number of the *incertas* or *schaufussi* workers were killed. In one of the *schaufussi* colonies the *lucidus* queen killed every one of the fifteen workers, leaving only the *schaufussi* queen. Later on, however, the *lucidus* queen herself died. My results agree with those of Wheeler mentioned above in that I did not see a queen at any time pay the slightest attention to the brood, her behavior being different in this respect from *F. sanguinea*, which, as Wheeler has shown, kills off the workers, takes possession of the brood and frees the first callows from their pupal envelopes. I will give a few notes from the two experiments in which the queens were adopted and are still living.

B. 23*a*.

Aug. 18—2.00 P.M. I place a dealated queen of *P. lucidus* in Petri dish with two dozen workers and 8 pupæ of *F. incerta*. They attack her at once. She tries to escape from them and usually manages to do so as she is very vigorous. She does not attack them, and bites them only when she cannot get away.

2.20 P.M. 3 workers holding her, one by a leg and one by an antenna.

4.00 P.M. Still being held by 2 workers.

5.00 P.M. The same.

6.00 P.M. The same.

Aug. 19—8.20 A.M. 3 workers holding her on the sponge. This hostility was continued against the queen until the morning of Aug. 23 when I found her dead.

Aug. 23—1.45 P.M. I place in another *Polyergus* queen. All but one of the cocoons have hatched. Some of the workers escaped, but two have been killed. There are now 15 workers in the nest and 1 of them is injured. The injured one attacks the queen and is soon killed by her. None of the other workers attempt to attack the queen. She runs about in the nest and attempts to escape.

4.00 P.M. 3 workers holding her, 2 by the antennæ and 1 by a leg.

4.30 P.M. 3 workers are licking her.

6.00 P.M. I worker is holding her by a leg.

Aug. 24—8.00 A.M. 2 workers are standing by her side; they do not attack her and she does not avoid them.

3.30. 1 of the workers is licking her.

4.00 P.M. 3 workers are licking her. A few minutes later one of them holds her by the tarsus for a while.

Aug. 25—8.00 A.M. The *Polyergus* queen is dead.

2.45 P.M. I place in another queen.

Aug. 26—9.00 A.M. She is standing in the midst of the workers and seems to be entirely uninjured. The workers do not attack her. There are several dead workers in the nest.

11.45 A.M. She moves around with the workers and does not try to avoid them, nor do they avoid her nor show any hostility to her.

Aug. 27—8.00 A.M. The workers are standing all around her; there are 10 left alive.

Sept. 12. I have examined the nest several times every day up to the present time and have never seen her attacked. She has been fully adopted.

B. 23*b*.

Aug. 19—11.20 A.M. I place a *Polyergus* queen in one chamber of a nest containing the mother queen, 16 workers and a few larvæ and pupæ taken from a large colony of *F. incerta*. The *Polyergus* queen runs about in the nest trying to escape. The workers are afraid of her and only a few try to attack her. They gather up their pupæ as they would in case of a raid.

1.45 P.M. All but 6 of the workers escape past the cotton plug into the other chamber.

6.00 P.M. The *Polyergus* queen is standing by herself in a corner of the nest The *incerta* queen and workers are standing in another corner with the brood.

Aug. 20—8.00 A.M. The same.

2.00 P.M. The same.

Aug. 21—10.30 A.M. The same. The *Polyergus* queen kept aloof from the *incertas* all the time until August 24. When I examined the nest at 1.00 o'clock on that day the *Polyergus* queen was running around in the nest but avoiding the *incertas* and they seemed afraid to attack her. About an hour later, however, I found the *incerta* queen dead and the *Polyergus* queen standing near her. I removed the dead queen and examined her. I found four cases where her thorax had been punctured by the sharp mandibles of the *Polyergus* queen. From that time on the *incerta* workers not only did not attack the *Polyergus* queen but they ceased to avoid her, and and on the following day I found one of the workers licking her. Since then they have treated her as they treated their own queen before. There are 8 workers in the nest now.

Besides the experiments with the above mentioned ants I tried a few adoption experiments with queens of *F. nepticula*, *F. sanguinea* var. *rubicunda*, and one with a queen of *F. difficilis* var. *consocians*.

The queens of *F. nepticula* were tried with small colonies of *F. inserta*, *F. fusca* var. *subænescens* and *F. subpolita* var. *neogagates*. The two queens that I tried with *incerta* and *subænescens* gave negative results. The workers attacked the queens fiercely in each case. The active little queen defended herself, however, by seizing them with her mandibles. The movements made the first few minutes were so rapid that the eye could scarcely follow them. In an hour or so, however, the queen was killed in each nest. The behavior was the same when I placed a queen in with a small colony of *F. subpolita*, but the *subpolita* workers being smaller than those of the other two species were not able to kill the queen so quickly, and after a fierce struggle of a few minutes she escaped from them. Although she was attacked again from time to time the attacks were not so fierce and several times I saw workers licking her; not only that, but her behavior then became more like what Wheeler has described for the queens of *F. consocians* when introduced into a colony of *F. incerta*, more insinuating and conciliatory, and twice I found the *nepticula* queen feeding a *subpolita* worker. Although the two queens which I tried with this *subpolita* colony were both finally killed I think the behavior both on the part of the queen and the workers tends to confirm the conclusions reached by Wheeler in 1906, that *F. nepticula* is a temporary parasite and that its probable host is *F. subpolita*.

The four queens of *F. sanguinea* var. *rubicunda* which I tried with small colonies of *F. subsericea* were not in very good condition as I had kept them too long in confinement without food and with one exception were killed. This one, however, behaved as did those in the experiments described by Wheeler in 1906. She killed four of the eight workers in the nest and after some time took charge of the pupæ. The other four workers remained hostile for about two weeks, after which they adopted her and helped take care of the brood.

The one colony of *incerta* with which I tried a queen of *F. consocians* contained the mother queen, about three dozen workers and several cocoons. I placed the *consocians* queen in with them on August 5 at 4.30 P.M. The introduction of the queen caused very little disturbance. The workers she met nabbed at her and pulled her legs and antennæ a little but not at all violently. Within an hour after being placed in she was fully adopted and was going about feeding the workers by regurgitation. The two queens, *incerta* and *consocians*, lived peacefully side by side. I still have the colony in the laboratory, September 16, and the yellow *consocians* queen seems to be perfectly happy with her strange nest mates. While on a field trip with Professor Wheeler near Colebrook, Conn., July 30 and 31, he found two mixed colonies of *incerta* and *consocians* and has given me the following notes which he made of them:

"Colebrook, Conn., July 30, 1910.

"No. 1. A mixed colony (in the second year) consisting of a *consocians* female and about 100 workers with brood, and a somewhat smaller number of workers of *incerta*. This was under a stone on Mt. Pisgah.

"No. 2. Colebrook, Conn., July 31, 1910. At Beech Hill near the Massachusetts boundary north of Colebrook I found a large and flourishing colony of *F. incerta* containing fully 200 workers and much brood, containing a single *consocians* queen that must have been very recently adopted. This is the largest *inserta colony* in which I have found a *consocians* queen."

The finding of mixed colonies of these two species, *incerta* and *consocians*, near Colebrook, Conn., a number of years ago and subsequent extensive field observations and experiments is what led to the discovery by Wheeler of the phenomenon of temporary parasitism among ants. At the same time he also showed that temporary parasitism exists in other American ants and predicted that it would be found to exist in certain European ants. These predictions have since been verified by a number of European myrmecologists. The results of the experiments recorded in this paper verify his predictions concerning the queens of *A. tennesseensis*, *L. latipes*, *L. umbratus minutus* and show that *F. obscuriventris* is a temporary parasite upon *F. subsericea* just as he has shown that the European *F. rufa* is parasitic upon the typical *F. fusca*.[1]

A question which will naturally arise in the minds of most people is, "To what extent would it be possible to secure the adoption of non-parasitic queens by workers of different species or even of different colonies of the same species?" Much light would be thrown on the whole subject if an extensive series of experiments with such queens should be conducted, and in the future I hope to be able to perform such experiments. Also, the logician will say that it should not only be proved that queens that are supposed to be temporary parasites may be adopted by workers of another species but it should also be proved that such queens are incapable of founding colonies unaided. That such is the case with some of the queens experimented upon we have only such negative evidence as I have given above, and positive evidence, one way or the other, can be obtained only by an extensive series of careful experiments. However, positive evidence that any of the queens given above as temporary parasites are able to establish colonies independently would not necessarily prove that they may not also be in part temporarily parasitic upon some other species. It is more than likely that a very large number, perhaps most of the ants are in some stage of development toward parasitism. For instance, the above experiments show that the queens of *F. obscuriventris* are not so easily adopted as those of *F. consocians* but much more easily than those of *F. nepticula* or of *A. tennesseensis*. Wheeler has shown that the first step toward both temporary and permanent parasitism from the primitive independent type of colony formation is the facultative adoption of the queen by workers of the same species, the second the obligatory adoption of the queen by workers of the same species, and third the obligatory adoption of the queen by workers of another species. Perhaps the last species in which one would look for traces of even the first steps toward parasitism would be our dominant species *L. niger* var. *americanus*, since it is well known that the queens of this species are able to extablish colonies independently. Workers from one colony of this species are always very hostile to those belonging to another colony and still more so towards queens from another colony, yet, out of eight attempts I succeeded in getting one young fertilized and dealated queen adopted by a colony of *L. americanus* consisting of about three dozen workers, six cocoons and a number of very small larvæ. It is therefore very likely that many of the queens of *americanus* establish their colonies with the aid of workers either of the same or of a different colony. Thus we see that even in our most dominant species we may find the first two steps toward parasitism. It is easily seen, therefore, that there is still a vast amount of work to be done before the last word can be said upon the interrelations of the different species of ants.

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[1] "Observations on some European Ants," 1909.

In conclusion I wish to thank Professor W. M. Wheeler, under whose direction the work was done, for his many helpful suggestions while the experiments were being conducted and for reviewing my manuscript.[1]

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[1] The colonies containing the adopted queens were left at the Bussey Institution and Mr. J. W. Chapman, who was kind enough to care for them, wrote me on November 25, that in each case the adopted queen was treated just as though she were the rightful queen, so that there is no question but that the adoption was complete.

M. C. T.

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BIOLOGICAL BULLETIN

EXPERIMENTAL CONTROL OF MORPHOGENESIS IN THE REGULATION OF PLANARIA.

C. M. CHILD.

The present paper is a brief account of certain results of experimental work upon *Planaria* which has extended with interruptions over the last ten years. The data presented here concern *Planaria dorotocephala* Woodworth, but *P. maculata* is similar in most respects. A complete account of the work will appear elsewhere.

1. The Dominance of the Anterior Region in Regulation.

My experiments support very positively the conclusion that in *P. dorotocephala* the anterior region, and more specificially the head region, controls the process of regulation in regions posterior to it and within a certain distance, *i. e.*, it is physiologically dominant over these regions. A limit of effectiveness for this dominance exists and this varies in general with the rate or intensity of the processes in the anterior region. Moreover, not only is the head region dominant over the regions posterior to it and within the limit of effectiveness, but in general a given level of the body is dominant over regions posterior to it within the limit and is dominated by regions anterior to it.

Before considering other facts it is necessary to recall that in nature all asexual individuals of *P. dorocephala* above a certain size consist of at least two zooids and the larger animals of more than two. This is shown by the following facts: (1) If the animals are cut into a series of small pieces of equal length from the anterior end posteriorly, the capacity for head formation decreases and disappears in successive pieces from the anterior end to about the middle of the postpharyngeal region, *i. e.*, the level where fission occurs, and then increases suddenly. This sudden increase represents the anterior region of the second zooid. Where the second zooid is of considerable length a similar decrease in the capacity for head formation occurs from its anterior end to about the posterior one half to one third of its length, where again a sudden increase in the ability of the pieces to form heads occurs. Posterior to this level there is a more or less diffuse region of very great capacity for head formation probably in reality a series of head regions, extending to the end of the body. Other features of regulation, the rate, the relation between regeneration and redifferentiation, the size of the head and the position of the pharynx show corresponding regional changes. Most of these facts were briefly stated in an earlier paper (Child, '06*b*) and will be considered more fully elsewhere.

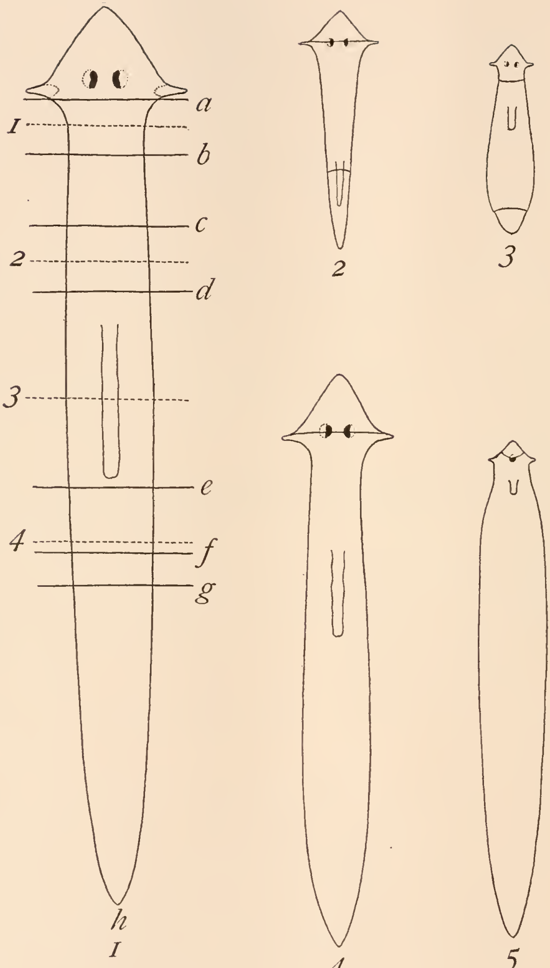
Secondly, it is possible by various means to induce fission in a very short time in animals which are far below the size at which fission would occur in nature, or which for other reasons would be incapable of fission under natural conditions. In all cases where fission can be induced it occurs at a level where sudden increase in the capacity for head formation appears. Some of the methods of inducing fission have been described in another paper (Child, '10*b*).

And finally, the length of the second zooid, together with other zooids which may arise from its posterior region varies with the length of the whole, but not proportionally. In very small animals the second zooid cannot be found by any methods thus far employed, but as the animal grows longer, it appears at the posterior end as a short region of high capacity for head formation and as growth continues it increases in length more rapidly than other parts; consequently in very large animals the postpharyngeal region is often double or more than double the length of the prepharyngeal, while in very small animals the prepharyngeal region is the longer. In *Planaria* the second zooid does not develop a head as visibly differentiated region before its separation, as do the posterior zooids of the Microstomidæ and annelids. This is because the processes which make this region morphologically posterior are more firmly fixed in *Planaria* than in those forms. So long as it remains attached to more anterior regions, the development of the second zooid can proceed only to a certain stage because existing conditions inhibit it. But, as I have pointed out, this development does proceed far enough to permit some degree of independent motor reaction in the second zooid, and it is this which brings about fission.

From the fact of the existence of the second zooid it follows that the region which represents physiologically the posterior end of the first zooid is somewhere near the middle of the postpharyngeal region, or in very large animals, in its anterior third. If we wish to compare anterior and posterior regions of the same zooid, this fact must be kept in mind. Turning now to the question of the dominance of the head region, the facts which indicate this dominance are briefly as follows: A piece above a certain size from any level of the body is capable of reproducing all parts which lie posterior to its level whether it produces a head or not, but it is incapable of giving rise to any of the parts which lie anterior to its level, unless some approach to head formation occurs first.

For example, a piece like *cd*, Fig. 1, is capable of producing a new pharynx and the characteristic postpharyngeal intestinal branches, even though it may fail to form a head, as is the case under certain conditions. On the other hand the piece *ef*, from the postpharyngeal region, *i. e.*, the posterior region of the first zooid, never produces even a pharynx unless some approach to head formation occurs first. If it remains headless as it usually does, it also remains without a pharynx or mouth and no prepharyngeal intestinal region with an axial intestine ever appears. Anterior regulation in such cases is limited practically to closure of the wound.

The dominance of the head region is also clearly shown by the relation between the rapidity of development and the size of the head on the one hand and the position of the pharynx on the other. In pieces from near the anterior end (*e. g.*, *ac* or 12, Fig. 1) the head is disproportionately large and develops very rapidly and the pharynx is situated near the posterior end of the piece (Fig. 3), while in pieces such as *eg*, Fig. 1, from the posterior part of the first zooid, the head is relatively small and forms slowly and the pharynx is anterior to the middle of the piece, Fig. 3. Moreover, it is possible to demonstrate experimentally in pieces



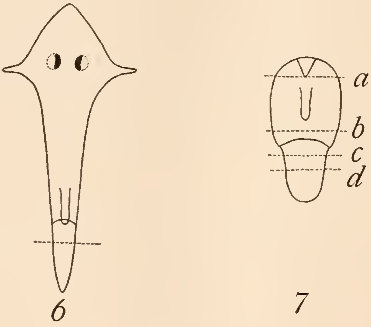
Figs. 1–5.

from the postpharyngeal region that any external conditions which alter quantitatively the processes which give rise to a head also alter the position of the pharynx. A single example is given here to illustrate this relation. The piece *eh*, Fig. 1, including the whole postpharyngeal region and the second zooid, always gives rise, under anything like natural conditions, to animals like Fig. 4 with large heads, normal eyes and the pharynx somewhat anterior to the middle. If, however, we decrease the rate of the metabolic processes by means of low temperature, anesthetics, CO_2, etc., the head forms much more slowly, remains much smaller, often possessses only a single median eye and the pharynx appears close behind the head and remains of small size. Fig. 5 shows the position of the pharynx in such a piece after regulation in 1.5 per cent. alcohol, 0.3 per cent. ether or in water containing CO_2. By varying the external conditions quantitatively all gradations between the conditions of Figs. 4 and 5 can be obtained. Changes in the other direction can be brought about by high temperature and also by other conditions which increase the rate of reactions in the body. In such cases the head is larger and develops more rapidly and the pharynx lies further posteriorly.

In general I have found it possible by these and other methods to alter the localization of the pharynx in pieces within very wide limits. Since the pharynx is situated at the posterior end of the median axial intestinal branch of the prepharyngeal region, the localization of the pharynx in the piece is directly related to the length of this axial intestine. In these experiments a definite spatial relation between the rate of the dynamic processes and the localization and size of organs and regions appears.

Moreover, the dominance of the head region and the limit of its effectiveness also appear in the conditions determining the origin of a second zooid. In newly hatched *P. maculata*—I have not as yet had opportunity to examine newly hatched *P. dorotocephala*, but have no doubt that they are similar—the second zooid is not present and even the whole postpharyngeal region is incapable of forming a head when isolated. In this respect it is like the posterior region of the first zooid in larger animals. As the worm grows longer the capacity to produce a head appears posterior to a certain level, and once established, the second zooid grows nore rapidly than the first. When the animal is 5–8 mm. in length a very short second zooid is present and in animals sightly longer than this fission may be induced experimentally (Child, '10*b*). In the posterior region of the second zooid a third zooid arises early because the head region of the second zooid is not sufficiently active to control more than a short region; the extreme posterior region of the body often seems to consist of a series of very short head regions, reminding one of the series of minute segments which often appear in the growing posterior region of various annelids.

Under experimental conditions it is possible to delay or inhibit the appearance of the second zooid in regulating pieces or to accelerate it. For example, if we isolate the region anterior



Figs. 6–7.

to the level *c* or 2 in Fig. 1, including the old head, it forms an animal more or less like Fig. 6, in which the head and prepharyngeal region are disproportionately large. If now after the growth and differentiation of the posterior new tissue is completed, we isolate the posterior end at the level indicated by the dotted line in Fig. 6, we find it does not produce a new head, *i. e.*, it is physiologically as well as morphologically a posterior end. If we feed pieces like Fig. 6 so that growth occurs, we shall find that after they attain a certain length the second zooid appears and after this the posterior region of the animal shows a high capacity for head formation. But such animals must grow to a much greater length than young normal animals before the second zooid appears, because in them the dominance of the head region is effective over a greater distance than in young aninals, and a sufficient degree of physiological isolation (Child, '10*b*, '11*a*) of the posterior region does not occur until a greater length is attained

On the other hand, in pieces of *Planaria* which remain headless second, third and further zooids arise at once and if the pieces can be induced by stimulation to move about sufficiently, fission often occurs. The headless piece, unless very small, always produces a very large amount of new tissue at its posterior end (Fig. 7). This new tissue consists of new zooids; if we cut it off at any of the levels *b*, *c*, *d* (Fig. 7) we find that it always produces a normal whole very rapidly. If we remove the anterior end of the headless piece at the level *a* in Fig. 7 after the second zooid has formed at its posterior end we find that it is now capable of forming a normal head, but this is possible *only when the second zooid is present*, for if we remove both the anterior end and the second zooid, the remaining piece is incapable of forming a head. This last experiment as well as others to be described elsewhere shows that the second zooid influences regions anterior as well as those posterior to its anterior end.

In general any condition which retards or inhibits the development of the head or the dynamic processes in the developed head favors the development of a second zooid at the posterior end and any conditions which accelerate the dynamic processes in the anterior region retard the development of the second zooid. As noted above, the length which the animal attains before it gives rise to a second zooid may also be varied experimentally. In these experiments we have a demonstration of the spatial limit of effectiveness of physiological correlation and of the relation between this limit and the rate or intensity of dynamic processes in the region of origin.

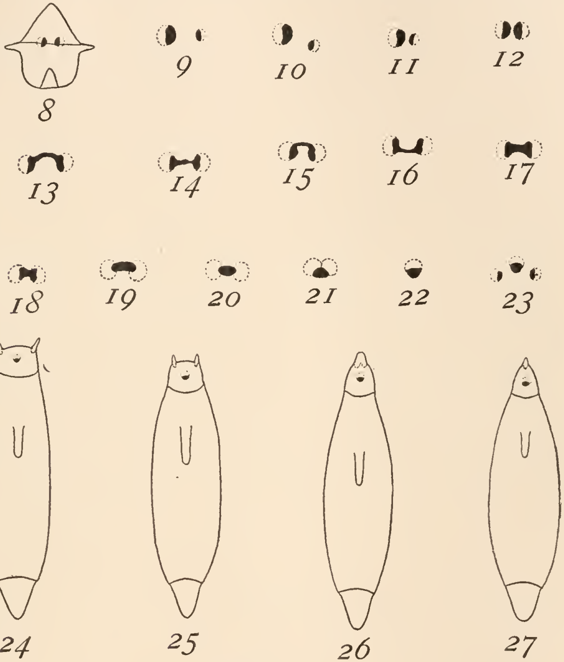
One other point may be added as indicating the dominance of the head region. In animals which decrease in size in consequence of starvation the head region decreases less rapidly than other parts and so becomes relatively larger the further the reduction proceeds. Manifestly the head region is able to live largely at the expense of other parts, to use their substance in maintaining its own structure.

Only a few few brief suggestions as to the occurrence and general significance of dominant and subordinate regions in organisms are possible here. The dominance of the growing tip over other regions in the plant is a well established fact: in my work on *Tubularia* (Child, '07*a*, *b*, *c*, *d*) I called attention to various facts which indicate that the hydranth region is physiologically dominant over other parts and the same is true for *Corymorpha* and many other, perhaps all hydroids and for all actinians with which I have worked. Moreover, when we recall that in most if not all animals development of the egg begins at the animal pole and that the distal or anterior region arises at or near this pole, the possibility that the "animal" distal or anterior region is very generally dominant becomes at once apparent. I believe that we have here a very general law of development which has not been clearly recognized. It is probable, however, that in many forms the specification of different regions of the egg becomes fixed to such an extent that their further differentiation is to a greater or less extent constitutional rather than correlative, but there are various facts which indicate that within each such "self-differentiating" part we shall find a condition of dominance and subordination of parts more or less similar to that which exists in the whole egg and often even in the adult of some other less highly differentiated forms.

II. THE REGULATORY DEVELOPMENT OF THE ANTERIOR END.

The "normal" head of *P. dorotocephala* possesses the form shown in Fig. 1. As in various other species of *Planaria* the eyes consist of black pigment spots and unpigmented sensory areas. The sensory cephalic lobes or auricles are also only slightly pigmented and are marked off as well-defined light areas from the rest of the dorsal surface of the head, which is dark brown. The light areas of the eyes and the auricles are indicated in Fig. 1 by dotted lines.

The result of regulation in the larger isolated pieces is usually a "normal whole" (Fig. 4), *i. e.*, and individual with head essentially like Fig. 1, though varying in size, and the other organs characteristic of the species in nature. In pieces below a certain size which varies with the region of the body and with other internal and external conditions, normal wholes develop from isolated pieces but rarely or not at all. A brief description of some of the characteristic results of regulation under these conditions is necessary. For convenience these are arranged under a number of heads, but it must be understood that the "types" thus distinguished are by no means sharply defined. They grade into each other in various ways, so that the whole series of types is actually a continuous series.

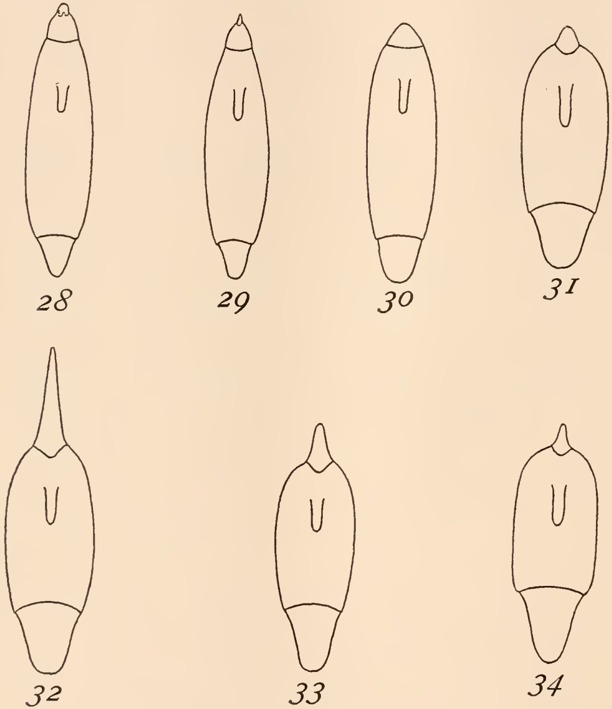


Figs. 8–27.

1. *Tailless.*—(Fig. 8.) Very short pieces from the more anterior regions often give rise, especially under certain experi-

mental conditions to heads without pharyngeal or postpharyngeal regions.

2. *Normal.*—(Figs. 2, 3, 4, 6.) In larger pieces these are all much alike (Fig. 4), but in smaller pieces they differ according to the region of the body and other internal and external factors.



Figs. 29–34.

Short pieces from the anterior region produce normal wholes like Fig. 2, those above a certain size from the pharyngeal region or immediately posterior to it produce wholes like Fig. 3.

3. *Teratophthalmic.*—(Figs. 9–23.) Abnormalities of the eyes are frequent in regulation of pieces of *Planaria* and their occur-

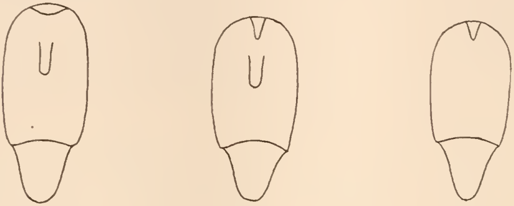
rence has been noted by various authors, though no one has previously attempted to determine the conditions of their appearance. They are of various kinds, but in most cases fall into one of the following groups: differences in size or asymmetries of position (Figs. 9–11); approach to the median line (Figs. 11–12) partial union of pigment areas (Figs. 13–19); single median pigment areas with double sensory areas (Figs. 20–21); single median eyes (Fig. 22); three eyes, one median, others right and left (Fig. 23). In all of these cases the head may be entirely normal otherwise, though the more extreme types of abnormal eyes (*e. g.*, Figs. 20–22) usually occur in heads of relatively small size and slow rate of development.

4. *Teratomorphic.*—(Figs. 24–27.) Very frequently, however, the head itself is abnormal in form, the median anterior region being incompletely developed or absent and the auricles appearing on the anterior end of the head and in various degrees of approach to the median line: they may be separated by a considerable interval (Figs. 24, 25), or partially (Fig. 26) or completely united in the median line (Figs. 27). As soon as the new heads become pigmented the unpigmented auricular areas are clearly visible and in such cases as Figs. 26 and 27 it is these areas which enable us to identify with certainty the auricles. Teratomorphic heads almost invariably possess only a single median eye, or they develop a single median eye first and later two more symmetrically placed eyes (Fig. 23); they are always of small size and develop slowly.

5. *Anophthalmic.*—(Figs. 28–34.) These pieces show a distinct outgrowth of new tissue at the anterior end, varying widely in form, but always without eyes. This outgrowth is well innervated and its motor activities resemble those of a head. Sometimes the partially or wholly fused auricles appear anteriorly in the median line on the outgrowth (Figs. 28, 29), as in the extreme type of teratophthalmic pieces (Figs. 26, 27), but usually the outgrowth shows no visible differentiations characteristic of a head. In shape the outgrowth ranges from a broad and blunt form like Figs. 30 and 31 to a very slender form of varying length, often almost as long as the remainder of the piece (Figs. 32–34). These pieces always give rise to a new

pharynx whether they arise from the old prepharyngeal, pharyngeal or postpharyngeal regions.

The anophthalmic pieces are always more active than the headless pieces described below and their movements are better coordinated; moreover, the motor activity of the anterior outgrowth itself shows very clearly that it represents some approach to head formation.



35 36 37

FIGS. 35-37.

6. *Headless.*—(Figs. 35–37.) The headless pieces are distinguished from the anophthalmic by the fact that the new tissue merely fills the contracted wound and does not grow out. According to the degree of contraction of the wound these pieces may appear like Fig. 35 or Fig. 36. Headless pieces which arise from the old prepharyngeal or pharyngeal region always develop a new pharynx (Figs. 35, 36), but these from the postpharyngeal region do not give rise to a pharynx (Fig. 37).

It will be observed that in the order figured types 2–6 present a gradation from the normal head to the headless condition. The eyes first show irregularities, then appear nearer together and finally in partial or complete fusion in the median line. In the one eyed forms the region between the auricles may be reduced or absent and the auricles appear in various degrees of approach to each other, or like the eyes partially or wholly united in the median line, and this latter condition is sometimes seen in the anophthalmic forms: in these all possible gradations appear between an outgrowth resembling a head in form to a long slender pointed outgrowth or one which is short and small, and finally this condition passes into the completely headless condition.

The similarity of the first part of this series to the abnormal and cyclopian fish embryos obtained by Stockard is at once apparent.

III. EXPERIMENTAL CONTROL OF THE CHARATER OF THE ANTERIOR END.

Attempts to control the appearance of the various types of anterior end were first made in my experiments some five years ago and it was found that various methods of control were possible. The anesthetics were first used for this purpose almost three years ago; a brief report of certain phases of this work has already appeared (Child, '10*a*). Thus far I have been able to control the process of head formation through various internal and external factors.

In connection with these experiments it has become necessary to standardize the material as far as possible, *i. e.*, to obtain some method of comparing the physiological condition of worms of different size and age, of well fed and starved animals, of animals before and after regulation, pieces from different regions, etc. By the use of alcohol of low concentration (in most cases 1.5 per cent.) I have been able to accomplish this to a considerable extent. A part of the results of this work in their bearing upon the problem of senescence have been described elsewhere (Child, 11*b*). These experiments confirm the conclusion reached from experiments on regulation alone, viz., that in general the capacity for head formation stands in close relation to rate of the metabolic processes in the piece.

For given conditions a certain rate of reaction is necessary for the formation of a normal head. When the rate falls below this critical level teratophthalmic heads appear first, then as the rate falls still further the teratomorphic and anophthalmic and headless forms appear in succession. Moreover, among the teratophthalmic forms the cases of partial fusion represent decrease in rate below the critical level and the single median eyes further decrease. The irregularities of form and position occur most frequently at levels near the old head in small worms and in small pieces from large worms.

Since length of the piece, region of the body and physiological condition are all factors in the capacity for head formation, pieces which are to be directly compared must be of the same size, except of course where the size factors are under consideration, they must be taken from the same region of the body except where the regional factor is to be analyzed and from individuals in similar physiological condition except where different physiological conditions are being compared. As noted above a relative measure of physiological condition is possible; it is also possible to control the size of the piece and the region of the body from which it comes within certain limits, especially in the pharyngeal region and near the head where localized morphological landmarks exist.

The method of experiment which I have found most satisfactory is to compare considerable numbers of similar pieces rather than individuals. For a temperature experiment for example, animals of the same size and as nearly as possible in the same physiological condition are taken as the basis: from these pieces as nearly as possible of the same size and from the same region of the body are cut and a number, usually fifty, of these pieces is placed in one temperature and a like number in another. For an alcohol experiment fifty pieces obtained in a similar manner are placed in alcohol and fifty in water at the same temperature and under otherwise similar conditions.

By taking into consideration the factors of size of piece and region of the body it is possible to cut series of pieces which will give 100 per cent. or very nearly of normal heads, or on the other hand 100 per cent. or very nearly of headless forms, or we can obtain series which in consequence of unavoidable differences in size and region and differences in the physiological condition of the animals from which they were taken, produce a certain percentage of several of the types described above. In all these cases we can compare the results under the control conditions with those occurring under other definitely known conditions.

For example, pieces above a certain size from the anterior region of the body, which give 100 per cent. or nearly of normal heads in well aerated water at a temperature of 20°C. show a large percentage of teratophthalmic forms in water at 10°C. or in water containing CO_2 or in alcohol 1.5 per cent. at the same temperature as the control, and under more extreme conditions the teratomorphic, anophthalmic and headless forms appear.

On the other hand, if we take pieces which show nearly 100 per cent. of headless forms in well aerated water at 20° C. we find that similar pieces kept at 30° C. will produce not only anophthalmic, teratomorphic and teratophthalmic heads, but a considerable percentage of normal heads. These illustrations will serve to indicate the general character of the experiments along this line.

Below are given the results of a few series of experiments on size, region, temperature, alcohol, nutrition and mechanical stimulation.

1. *Size and Region.*

We may investigate these factors by cutting the whole body of worms of the same size and similar condition into one-fourth, one-sixth, one-eighth, one-twelfth and one-sixteenth pieces, etc., and comparing the results. The following table is a part of a series of this kind and shows in somewhat abbreviated form the results for one-eighth and one-sixteenth pieces of large worms. The head is not included and the pieces of the body are numbered consecutively 1, 2, 3, etc., from the anterior end backward; the consecutive numbers in the second column refer to the pieces. The remaining columns show the percentages of each of the regulatory types for each set of pieces. In this series each set consisted of only ten pieces, but the results are almost as uniform as in much larger series.

In this table the factors of size and region appear clearly. We see that the capacity for forming normal wholes decreases posteriorly and then in the region of the second zooid increases again suddenly. The anterior ends of the one-eighth pieces respectively are at approximately the same levels as those of nos. 1, 3, 5, 7, 9, 11, 13, 15 of the one-sixteenth pieces and it is at once evident that the longer one-eighth pieces possess a greater capacity for head formation than the shorter one-sixteenth pieces.

We can obtain a more striking expression of this fact by comparing very long and very short pieces from similar worms cut with anterior ends at the same level. One of my series of this

		Tailless Heads.	Normal Wholes.	Teratophthalmic and Teratomorphic.	Anophthalmic and Headless.	Dead.
One-eighth Pieces	1		100			
	2		10	90		
	3			20	80	
	4			10	90	
	5			10	90	
	6		10	20	70	
	7		80	20		
	8		90	10		
One-sixteenth Pieces	1	70	30			
	2		30	60	10	
	3		10	30	60	
	4		10		80	10
	5				100	
	6				100	
	7				100	
	8				100	
	9				80	20
	10			10	70	20
	11				70	30
	12			20	60	20
	13		20		60	20
	14		40		50	10
	15		80	10	10	
	16		90	10		

sort is as follows: from large worms of equal size and similar conditions fifty pieces were cut including the whole postpharyngeal region (*eh*, Fig. 1); a second set of fifty short pieces from the anterior end of the postpharyngeal region (*ef*, Fig. 1), *i. e.*, with anterior ends at the same level as the larger pieces, was used for comparison. The following table gives the results in percentages.

	Normal.	Teratophthalmic.	Teratomorphic.	Anophthalmic.	Headless.	Dead
Long	100	0	0	0	0	0
Short	0	0	2	10	82	6

In the same way we may compare other levels of the body and show to what extent the power of head formation depends upon the cells near the anterior cut end and to what extent upon their connection with more posterior regions. My experiments along this line show that in general the ability to form heads in pieces from the posterior region of the first zooid is almost wholly dependent upon their connection with more posterior regions, while in pieces from the anterior end of the body it is very largely independent of such connection.

2. *Nutrition.*

My experiments along this line are rather extensive and the results are of considerable interest. I give here only a single series of experiments. The pieces used included approximately the region between the lines 2 and 3 in Fig. 1; fifty pieces were cut from worms which had been fed every 2–4 days for two months (I), another set of fifty pieces from worms of approximately the same size which had had no food for forty days before the experiment began (II). Results are given in percentages.

	Normal.	Teratophthalmic.	Teratomorphic.	Abophthalmic.	Headless.	Dead.
I.	4	46	6	30	14	0
II.	0	0	2	10	64	24

The capacity for head formation is manifestly much greater in the well-fed (I) than in the starved pieces (II).

3. *Temperature.*

Under this head only a single series is given, but the results are characteristic. In this series fifty pieces from similar worms including the region between the lines 3 and 4 in Fig. 1 were allowed to regulate at each temperature. Results are given in percentages.

	Normal.	Teratophthalmic.	Teratomorphic.	Anophthalmic.	Headless.	Head.
28°–30°.	74	22	2	0	0	2
18°–20.°.	22	40	2	14	22	0

The death of one individual (2 per cent.) at high temperature was purely accidental. The percentages show the general character of the results. With greater differences of temperature greater differences in the results can be obtained.

4. *Alcohol.*

The experiments with alcohol and other anæsthetics present certain complicating factors which will be considered fully elsewhere, but the effect of alcohol upon the capacity for head formation and upon the character of the head formed is readily demonstrated.

In the series presented here fifty pieces from similar worms, including the region between lines 2 and 3 in Fig. 1 were placed in alcohol 1.5 per cent. for 48 hours after section and then removed to water; fifty similar pieces were placed in water for control. Results are in percentages.

	Normal.	Teratophthalmic.	Teratomorphic.	Anophthalmic.	Headless.	Dead.
Alc. 48 hrs.	14	16	8	18	38	6
Water	20	44	2	26	8	0

A much larger percentage of headless forms appears in alcohol than in water.

5. *Complications in the Effects of Depressing Agents and Conditions.*

In the present paper I wish merely to record the fact that under certain conditions and within certain limits depressing factors increase the head-forming capacity in pieces, instead of decreasing it. As I shall show elsewhere, this result can be accounted for without difficulty. It depends largely upon the fact that the rate of metabolism in the cells at the anterior end of the piece which react to the wound increases in the course of this reaction, while that of more posterior regions of the piece remains essentially the same. The depressing factor decreases the rate in both regions and with a certain degree of depression the more posterior region will become almost quiescent while the anterior region which loses its previous differentiation in consequence of the wound reaction will still be active. Under these conditions the dominance of the anterior over the posterior region will be increased and a larger percentage of heads may be formed in spite of the depressing effect, simply because the anterior region is more capable of living, growing and differentiating at the expense of the posterior region. Under these conditions then the depressing effect will appear to be a morphogenic stimulus.

6. *Mechanical Stimulation.*

As is well known, the shorter pieces of the planarian body, especially those from the more posterior regions of the first zooid, very commonly move about but little until regulation has attained a certain stage; after this stage "spontaneous" movements occur. Larger pieces show these movements at earlier stages and in still larger pieces, *e. g.*, after removal of only the head region, they are never absent. In general the pieces which show more motor activity when left undisturbed in the dishes and which react more readily to slight stimuli are those which have the greater capacity for producing new heads, *i. e.*, for becoming wholes. From a number of pieces it is possible within a few hours after section to select with a considerable degree of certainty those which will form heads, or which will show the greater degree of head forming capacity, merely by observing the differences in motor activity and reaction to slight stimuli.

With these and various other facts in mind I carried out experiments to test the effect of mechanical stimulation to movement upon the formation of heads. The pieces were cut of such size and such distance from the old head that they showed little or no "spontaneous" movement during the first few days after section. Various methods of stimulation were employed: one of these was that of tilting the broad flat dish in which they were kept so that each piece was out of water for a few seconds. This usually serves to induce locomotion, but it does not continue long after the pieces are again in water. Sometimes the pieces were loosened from the glass by currents from a pipette or were turned upon their dorsal surfaces with needles, after which they usually righted themselves. But the most commonly used and most effective method was that of stroking or gently pushing the pieces with a small soft camel hair brush. Usually a few seconds of such stimulation was followed by relatively long sustained locomotion. The stimulation began a few moments after section of the pieces and was repeated at least every hour and often every half hour from about 8.30 A.M. till 6 P.M. and again at least twice between 8 and 11 P.M. each day until the heads in such pieces as produced heads were well developed and the eyes visible. Each time that the pieces of the one set were stimulated the water of the dish containing the control was gently disturbed and stirred in order to avoid as far as possible differences in aeration. Care being taken not to touch the control pieces directly, the movement of the water almost never induced movement of the pieces. The dishes were kept under as nearly as possible identical conditions of light and temperature.

Two series of experiments are given here. In the first the pieces included the region between the lines 2 and 3, Fig. 1, fifty pieces for stimulation (I) and fifty for control (II) being taken from similar worms.

	Normal.	Teratophthalmic.	Teratomorphic.	Anophthalmic.	Headless.	Dead.
I	0	28	8	32	32	0
II	0	8	8	26	58	0

The second series consists of pieces including the region between the lines 3 and 4 in Fig. 1 and from the same worms as the first. These pieces, being posterior to those of the first series and of approximately the same size produce heads less frequently. As in the first series, fifty pieces were used in the stimulated set (I) and fifty in the control (II).

	Normal.	Teratophthalmic.	Teratomorphic.	Anophthalmic.	Headless.	Dead.
I	4	10	2	18	64	2
II	0	6	2	12	80	0

The two pieces which produced normal heads in the stimulated set of this series were undoubtedly pieces which included the anterior end of the second zooid: the presence of this region even at the posterior end of a piece increases greatly its head-forming capacity. In both series the effect of stimulation appears unmistakably in the increased capacity for head formation. Without doubt it is not the actual performance of the movement itself but the stimulation which is the important factor. Stimulation means increase in the rate or intensity of the dynamic processes in the piece and this appears in increased and altered formative capacity. I believe that these and other similar experiments to be described elsewhere establish and confirm against all objections my position that the process of regulation is essentially a dynamic, or as I have often called it, a "functional" process or complex of processes.

7. *The Morphogenic Effect of Quantitative Changes in Conditions in General.*

In the temperature, nutrition and stimulation series the experimental conditions differ quantitatively from those of the control.

For reasons which I shall state later it seems probable that the factor of length of the piece is essentially quantitative in its effect upon head formation, and the regional factor is certainly very largely quantitative. The effect of alcohol and other anæsthetics is also quantitative, at least in large part.

The results obtained in these experiments point to the possibility of controlling and analyzing morphogenesis in a great variety of ways and of obtaining some insight into the nature of morphogenic processes themselves. As regards the data presented, there are many facts which indicate that the rate of oxidations or of certain oxidation processes is the chief factor in the results obtained.

From the morphological point of view the most important point is that certain organs may alter their localization, their form and their relation to each other and may finally disappear as the rate of the processes concerned decreases and vice versa. It is evident that under given conditions a certain rate of certain dynamic processes is necessary for the production of certain morphological effects.

IV. THE EFFECT OF CHANGE OF POSITION OF PARTS IN THE BODY.

If we cut small pieces from the posterior region of the first zooid such for example as *ef*, Fig. 1, they will give 100 per cent. or nearly of headless forms, If, however, we cut out a large piece, *eh* (Fig. 1) and allow a head to form at its anterior end and then after a week or ten days cut out small pieces just behind the head, we shall find that they now produce 100 per cent. or nearly of forms like Fig. 2, *i. e.*, normal wholes. These pieces represent approximately the region which when taken from the original animal gave rise to 100 per cent. or nearly of headless forms. Their capacity for forming heads has been widely altered by their changed position in the body and more specifically I believe by altered correlation with other parts. The cells of such a region are not directly concerned in the formation of a head, but the more anterior their position in the new individual, the greater their head forming capacity becomes.

In a similar manner we can decrease the head-forming capacity of parts which were originally near the anterior end and possessed the capacities characteristic of that region. We can make almost any portion of the prepharyngeal region into a pharyngeal region with corresponding change in regulatory capacity. The anterior region of the second zooid, with its very high regulatory capacity may be changed into the postpharyngeal region of a first zooid with a great decrease of regulatory capacity. Changes of this kind may be made almost at will merely by so arranging the experiment that the part in question is brought into a certain position with regard to other parts. It not only acquires a different structure but its capacities are altered. These experiments show a wide range of possibilities of influencing the morphogenic capacities of parts indirectly through correlation. I believe the point is of some general interest. In general terms these experiments show that the regulatory capacity of a part of the body may be altered through correlation. As regards a specific case, the head, they show that a part which when isolated originally possessed little or no capacity to form a head may have this capacity greatly increased by closer association with a region where a head is forming, even though the part itself is not directly concerned in the formation of the head. Such facts as these, possess, I believe, a certain significance in connection with the problem of inheritance. In these cases we actually alter the hereditary capacities of the part in question by altering its correlation with other parts.

Hull Zoological Laboratory,
University of Chicago,
March, 1911.

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THE BIOLOGY OF THE RED-BACKED SALAMANDER (PLETHODON CINEREUS ERYTHRONOTUS GREEN).

M. ETHEL COCHRAN.

Distribution.

Baird says, "Species of the genus *Plethodon* are found all across the North American continent." Boulenger makes the range of the subfamily Plethodontinæ North America, with possibly one species in the valley of the Rio de le Plata. Of the subfamily Plethodontinæ, Gadow states that it "is almost entirely American (with one species, *Spelerpes fuscus*, in Europe)," while Holmes says that "the Plethodontinæ form a large group which is mainly confined to America." In the last edition of his "Manual," Jordan gives the range of the family as "chiefly North America."

The "red-backed" salamander (*Plethodon cinereus erythronotus* Green) belongs to the Plethodontinæ and according to Jordan is confined to the eastern United States. Gadow says, "*Plethodon erythronotus* extends into Canada," while Boulenger would have it range in the eastern United States and Canada. Cope claims the species *Plethodon cinereus*, including all varieties, has an extreme range, being "found throughout the United States, east of the Mississippi River. It appears to be more abundant in the Middle States; its northern range is to the middle of Maine, Ontario and Michigan." Kingsley says it is the "most abundant salamander in the eastern United States."

Throughout Worcester County, Mass., this little salamander has proved very abundant. It is not an uncommon thing to find twenty or more during a short afternoon's walk. Every little wood has its dainty, shy, inhabitants who so easily may be overlooked.

Habitat.

The tiny creatures are not visible to the casual observer, for on bright days they are always concealed beneath stones or fallen logs. Holmes says they are in damp situations under rocks or decaying logs, while Montgomery found them near West Chester, Pa., "at all seasons, never in streams or boggy places, but in woods and hillsides beneath wood and stones, a strictly terrestrial species," Kingsley, on the other hand, claims "*Plethodon cinereus* is found everywhere in woods, under bark, logs and stone, in comparatively dry places." Miss Whipple speaks of its "being found far from any water supply."

Overturning a stone discloses a red-back at home. (Photograph by Dr. Miller.)

During a year's study of the species, they have been found in both damp and dry places, within five feet of a pond's edge and on rather dry, high slopes. In the daytime, they are always concealed. In one case, near a spring, a large sawdust pile that contained several planks furnished an abode for several salamanders.

One damp, dark day, when the rain was falling gently, three medium-sized individuals were found on the surface of the ground near stones from beneath which they had evidently come. In the same locality, five were found beneath stones; two were so placed that it seemed as if I had frightened them in before aware of their presence. This idea was strengthened by finding one of the two in an ant's nest, a thing not found before. Of several specimens kept in the laboratory, in vivaria, a few would venture out of their retreats on dark days.

Rarely, if ever, are the salamanders found in treeless places, but they seem to have equal preference for pine, birch or mixed woods. All slopes, if shaded and of not too sandy a nature, seem to provide suitable dwellings.

The natural crevices beneath stones are utilized by the red-backs. (Photographed by Miss Gulick.)

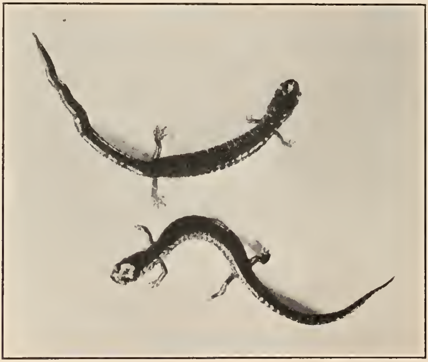
It seems that no holes are made by the salamanders themselves, but that they utilize whatever is at hand. Allen noted holes, but was not sure whether the salamanders made them or not. In a pile of stones or in a sawdust heap, there are many natural openings connecting in labrinthian fashion which are typical. In the vivaria kept in the laboratory, the pieces of moss were placed above a layer of sand and charcoal; a small dish of water was sunk to the level of the moss and the spaces between the pieces of moss, between the moss and sand, and between the "well" and sand were utilized by the red-backs as homes and highways.

In one instance, where two specimens were found in rather soft earth, a well-defined hole was followed—it led by a short gently-inclined path to a mass of rocks about a foot below ground where there were natural openings in all directions. The hole then appeared to be nothing but a natural opening worn smooth by use. Five other places were carefully studied where natural crevices seemed to be utilized.

The number of salamanders found under one object is variable; the most ever found was ten and countless times has only one been seen. There seems to be no evidence of their being or living in pairs during fall and early spring.

DESCRIPTION.

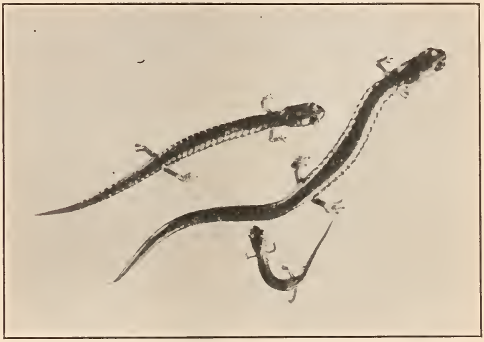
Kingsley calls these salamanders "the smallest in the United States." The largest specimen found measured 9.2 cm., while from 7.8 cm. seems to be the average length. The tail is about as long as the entire animal. Of two individuals, one, whose total length was 9.2 cm., had a tail 4.9 cm. long, while one 7.8 cm. long, had a tail 4.2 cm. in length. Jordan gives their total length as 3½ inches (9 cm.). The following measurements give some idea of the relative length and width of the body: total length, 8.4 cm., tail 3 cm., costals 2 cm., width of head 5 mm., width of body 4 mm., dorso-lateral diameter 2 mm.



Red-backs. (Natural size.) (Photographed by the author.)

They are slender, with an almost cylindric body and a sharply pointed cylindrical tail. The legs are thin and weak and the feet proportionately small. The anterior foot has three toes and an inner rudimentary one, while the posterior has four with one inner rudimentary toe. The body is not always lifted from the ground when the creature is walking—the tail never. *Spelerpes bilineatus* seems to be more slender than *erythronotus*, and when a dusky salamander (*Desmognathus fusca*) is about, the daintiness of a red-back makes it seem as clumsily built as an *Amblystoma.*

The most distinguishing feature of this species is the broad dorsal band of color that varies from bright, brownish-red to a sort of pale, dead-leaf brown. Age seems to make no difference in the color, nor has any sex differentiation been observed in fall and early spring. Two peculiar specimens found in company with several normal ones, in widely separated spots, were of a bright vermilion red, similar to the color of young *Diemyctylus*, over all the dorsal surface; the tail alone was a dark brown. It was probably a chance variation, as it seemed normal in every other respect.



Red-backs. (Natural size.) (Photographed by Dr. Miller.)

The sides of the body and much of the tail are of a dark gray-brown finely flecked with silver spots that grow more numerous toward the ventral side where the color is almost white, varied with a few darker spots. The ventral part of the tail and legs is much darker than the belly, while the dorsal surface of the legs is similar to the sides. The eyes are large for the size of the head, dark, iridescent, and very prominent. The nostrils are visible; the head is quite flat, the nose rounded, not pointed.

The skin is always moist and some amount of water is needed in the environment, for a specimen kept in a dry pasteboard box for a few hours was found in a much shriveled condition, dead.

The throat is in constant vibration which at times is very rapid; various counts were taken from seventy to one hundred and eighty a minute. The costals or costal folds, from sixteen to nineteen, are very easily seen. There seems to be no external character during fall and early spring to distinguish the sexes; in a few specimens dissected, the males were larger than the females.

Activities.

The red-backed salamander is almost entirely nocturnal. Jordan and Kingsley mention this fact, while of *Autodax lugubris* Hallow, which belongs to a genus close kin to *Plethodon*, Ritter says it is entirely terrestrial and nocturnal.

This salamander is quick of movement. Often when first discovered, it is found in a graceful, curled position, and seldom moves unless touched. If annoyed, it glides rapidly, sometimes into crevices or holes and sometimes into the moss or leaves, where it lies quietly hidden. In this rapid gliding movement, the tail is lashed from side to side, as if aiding the fragile feet and legs.

It is a great climber—individuals in the laboratory climb up the sides of a glass vivarium with great ease, using the moist, adhesive abdomen, as well as the feet and legs. They have been seen to do this on rather dark days as well as in the evening.

Kingsley mentions finding them out of doors on a "spear of grass or coiled at the apex of a rachis of a fern at a distance of from twelve to eighteen inches above ground."

There is a peculiar jumping habit common to the species—if one is held in the hand, a foot or more above the moss floor of a vivarium, it seems to gather itself together into a coil, and using its tail as an aid, it springs lightly to the moss. The whole process is indescribably quick. Kingsley says it "leaps by sudden unbending of the coiled body like some caterpillars."

The red-back's activities in the water are interesting. Jordan says it "rarely or never enters water." Miss Whipple has described it well: "Although certain lungless species may be more or less aquatic, their activities, even in water, are terrestrial. Various species will at first, when in an aquarium, swim to the surface, then around and around the edge of the aquarium as if seeking a means of escape, but at the instant active swimming ceases, the body sinks clumsily and heavily to the bottom, where they remain until disturbed or until another effort is made to escape. Lungless forms show on the whole little power to adapt themselves to aquatic life. Most are terrestrial in habit, some, *Plethodon cinereus* and *Plethodon glutinosus*, being found far from any water supply.

"The nares close as soon as the animal is submerged in water and remain so as long as the animal is in water. In a few cases, there were attempts at a feeble bucco-pharyngeal respiration, but even then the external nares were closed and water was drawn in and expelled through the slightly opened mouth."

Salamanders in the laboratory seem able to endure extreme cold, for in a vivarium the water was found with a thick coating of ice, but the salamanders were apparently unaffected.

The latest date of finding a salamander out of doors in the fall of 1909 was November 13. The first specimens this spring (1910) were found on March 20, on a warm, southern slope free from snow. At this time there was still much ice and snow in the woods all about. In midwinter, Montgomery says the salamanders are found deeper in the ground.

Food and Feeding Habits.

Regarding the frogs, salamanders, etc., Dr. Hodge says in "Nature Study and Life," "with one or two exceptions . . . they are all valuable insect destroyers, each for its peculiar haunts; they should be generally protected and utilized as beneficent forces in nature."

The red-back, like other amphibians, prefers live, moving food. On several occasions, bits of meat placed in the vivarium remained untouched for days and became covered with mould while if a piece were moved before a salamander, it was taken eagerly. Small wriggling earthworms are enjoyed. In one case, a small toad tadpole was offered to a salamander whose attention was so attracted at once by the struggles of the tadpole that it promptly ate it. A companion at the same time ate two tadpoles. Small insects are captured and eaten with avidity.

A moving object is noticed very quickly. The salamander will follow it fixedly for a moment, then, with gaze still upon it, creep slowly after until close at hand, when a rapid spring is made for it. Often it appears to have difficulty in swallowing food—the eyes close as several slow gulps are made and some time elapses before another portion is taken. No use of the hand in feeding has been noted, as is commonly seen in toads and frogs.

As a means of determining the kind of food taken in nature fifteen stomach-contents were examined. The salamanders came from several widely separated districts.

In general, specimens taken in the morning showed fuller stomachs than those taken in the afternoon.

The following specimens were found in the fifteen stomachs examined: Ants of several kinds, beetles of several kinds, bugs, caterpillars (cut-worm), centipedes, earthworms, fleas, flies of several kinds, maggots (rat-tail), midges, mites, mosquito wiggler, plant lice, plant remains, pseudo-scorpion, slugs (*Limax*), spiders, spring-tail (*Thysanura*), sow-bug, wasp-like insects.

Below is given a list of the contents of five stomachs that may be taken as typical.

A salamander captured May 17, 1910, at four P.M. in a sawdust heap near a spring, Auburn, Mass., contained: four snout beetles, one yellow ant, one small spider and ten mites.

A salamander found under a stone in an ants' nest, April 28, 1910, in the afternoon at Auburn, Mass., contained: one earthworm (2.75 cm. long), two mites, two small beetles, and three maggots that were probably young ants.

A salamdander found at Holden, Mass., at eight A.M., on April 27, 1910, contained: one earthworm (3.5 cm. long), one centipede, one black fly, one spider, one cutworm, one ichneumon maggot (probably from the cutworm), one pseudo scorpion, one sow-bug, and one unknown insect (which had eaten two small snails).

A salamander found on the afternoon of November, 11, 1909, at Gates Lane, Worcester, Mass., contained: three slugs (*Limax*, 1, 4, 5 mm. long respectively), one plant louse, one small worm, and plant remains (probably accidental).

A salamander captured in Norcross Woods, Worcester, Mass., at eight A. M., May 9, 1910, contained: one earthworm (2.2 cm. long), one brown spider (3 mm. long), two mites, one beetle, one spring-tail, one mosquito wiggler, one *Syrphus* fly, and plant remains.

Enemies.

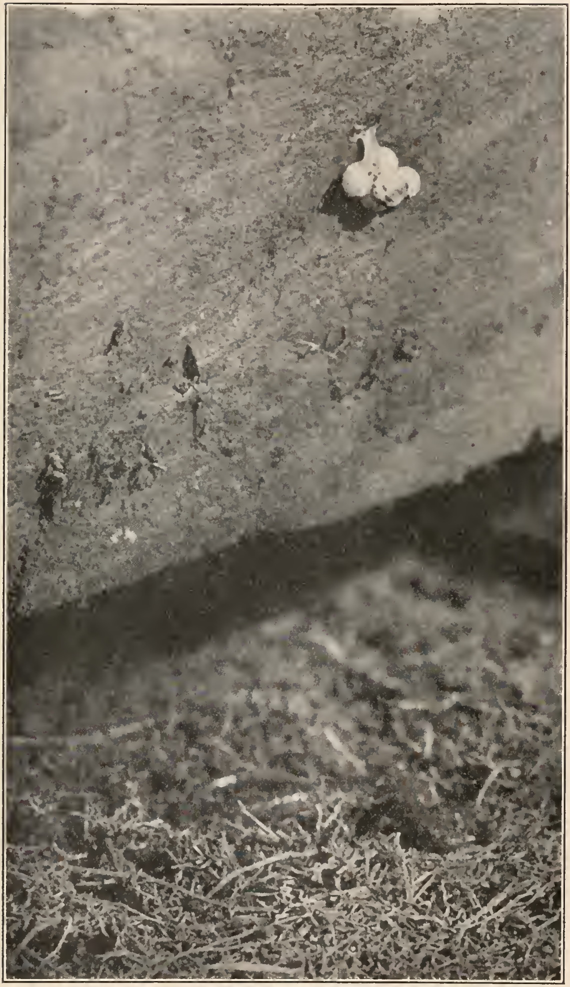
Snakes undoubtedly are great enemies of the salamanders. In a study made in Pennsylvania, Surface found that salamanders formed a large part of the diet of some snakes. The ribbon snake or striped garter snake (*Thamnophis saurita*), which belongs to the eastern United States and likes to live in shady, narrow, watered valleys, preys upon *beneficial* batrachians. "In four specimens containing food, four salamanders were found, two of which were *Plethodon cinereus;* 37.5 per cent. of the food of this species of snake was found to be salamanders." The common garter snake was found to destroy toads and salamanders among which was *Plethodon cinereus.* The water snake (*Natrix sipedon*) and the grass snake (*Liopeltis vernalis*) also were found to eat the red-back among other salamanders.

Ditmars states that the following snakes feed on salamanders, among which it is reasonable to include the red-back, as their haunts are much the same: ribbon snake (*Eutemia saurita* Linn.), mud snake (*Seminatrix pygæa*), brown or DeKay's snake (*Storeria dekayi* Hol.), green or grass snake (*Liopeltis vernalis*), eastern and western ringed-necked snakes (*Diadophis punctatus* Linn. and *D. amabilis* B. & G.).

A hungry, half-grown bull-frog ate a good-sized salamander with evident relish and a purple grackle treated one like a worm, beating it and breaking it with his bill before eating it.

Protective Devices.

Plethodon cinereus erythronotus, when annoyed, yields a colorless, glutinous secretion from its tail. A Californian salamander, *Plethodon oregonensis*, was found by Miss Hubbard to yield



An upturned stone, showing cluster of eggs, manner of attachment and brooding salamander. (Natural size.) (Photographed by Dr. Miller.)

on the tail an abundant poisonous fluid which did not prevent snakes from eating it. Easterly has written of the large granular glands of this salamander which are poisonous, but to what he does not state.

Baird speaks of the genus *Plethodon* whose skin exudes a highly glutinous secretion, but he makes no statement of its nature. Gadow says: "Numerous experiments have shown that the poison of toads, salamanders and newts is capable when injected, of killing mammals, birds, reptiles and even fishes, provided of course, the dose be proportionate to the size of the animal. Small birds and lizards succumb as a rule in a few minutes; guinea-pig, rabbits and dogs in less than an hour. This poison of amphibia is not septic, but acts upon the heart and central nervous system. Some authorities hold that the poison is an acid, others regard it as an alkaloid."

Raised stone showing pair of red-backs with eggs. (About one half natural size.) (Photographed by Dr. Miller.)

The fluid from a red-back's tail is very sticky when placed in the mouth; after a short time, a slight biting sensation is felt for a few moments.

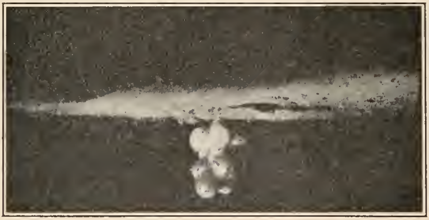
Several of the salamanders taken from their native haunts had every mark of possessing a regenerating tail. In one case, the tail had been cut or broken off nearly to the hind legs and the bud of a new one had grown out about a quarter of an inch.

Miss Towle found that *Plethodon cinereus* had the regenerative power also in the limbs.

Of *Plethodon oregonensis*, Miss Hubbard says it practices "autotomy only as a last desperate resource, and but in one region (directly behind the anus)." But when in two or three instances, the tail of a red-back has been held by forceps, the tail has come off very easily and in no particular region. Those found wild with incomplete tails appeared to have been broken in no particular joint. This salamander has not been observed thus far to snap or bite for protection.

INTELLIGENCE.

The red-backs are easily tamed, and will learn to eat food when offered on a splint. They object to being handled even after they have been in captivity a long time, probably because of the unpleasant warmth and dryness of the hand.



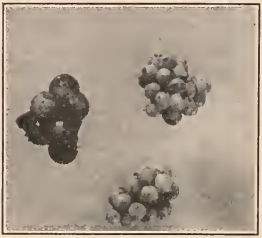
Cluster of eggs attached to a stone. (Two thirds natural size.) (Photographed by Dr. Miller.)

They appear to be very quick of hearing. Abbott, among other statements about the salamanders, mentions "their quickness of hearing." When undisturbed in their haunts, a whistle, clap of the hands, or a speaking voice sends them away. Abbott is of the impression that the salamanders give evidence of greater intelligence than the toads or frogs. His efforts to prove them possessed of cunning were not successful.

REPRODUCTIVE HABITS.

Baird writes of the genus *Plethodon*, "The eggs are deposited in packages or aggregations, in moist situations, under stones and logs, not however, in the water; and the larvæ lose their branchiæ at a very early age."

Montgomery describes one finding of eggs: There were five eggs under a stone in July with an adult female curled about on guard. The eggs were large, enclosed in gelatinous envelopes, and curled about a large, nearly spherical yolk mass.



Eggs of dusky salamander (two clusters at the right), and red back (one cluster at the left.) (Natural size.) (Photographed by the author.)

Miss Sampson says, "*Plethodon* lays its eggs on land."

Ritter and Miller describe the eggs of *Aurodax lugubris* Hallow, a Californian salamander closely related to *Plethodon*. They are attached singly to the under surface of stones. In one instance a female with fifteen eggs was found under a platform in front of a barn, in dry earth next the foundation wall. The eggs are laid in July, are about 6 mm. in diamaeter and hatch in fifty days.

During the summer of 1910, eggs of the red-backed salamander were found first on July 5. From then until the latter part of August, eggs were found. They are in the ordinary habitats beneath stones and logs. The stones are always those deeply set into the ground in close contact with it, probably because there the atmosphere is moist.

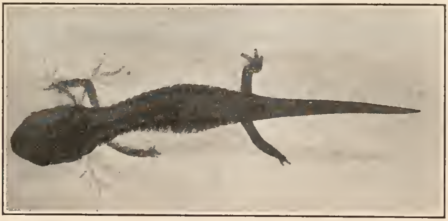
The eggs, from five to nine in number, are in grape-like clusters attached to the under surface of stones or logs, unlike those of *Spelerpes* and *Autodax lugubris* Hallow, which are attached singly. One pedicel supports a cluster. This pedicel is tough, white, and seems made of separate threads closely stuck together; it resembles the tough, outer membrane covering each egg. There is no unifrom arrangement of eggs in the cluster.

The egg of the red-back is spherical, 5 mm. in diameter, has a prominent yolk and considerable transparent jelly. On the outside is a thin, tough envelope to which particles of earth and leaves readily adhere.

The embryo is coiled about the yolk. None were seen in young enough stages to note the first appearance of color, but the red-brown of the dorsal stripe is visible at an early age.

Embryo red back, age unknown. (Enlarged about six times.) (Photographed by Dr. Miller.)

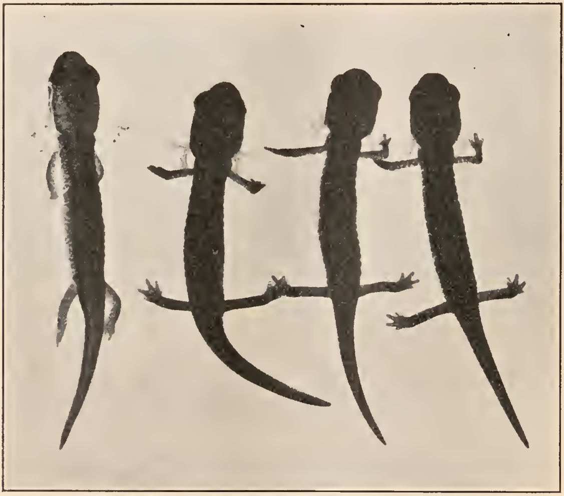
With each cluster of eggs, there was always one or two adults. Dissection showed that when there was one only, it was a female, when two, they were male and female. There is, accordingly, no evidence that the male takes any active part in the care of



Embryo red-back taken from egg some days before time for hatching, showing the three pairs of gills. (Enlarged three times.) (Photographed by the author.)

the eggs in this species. The egg cluster is always so attached that one of the adults may coil its body about it. Sometimes a crevice beside a rock is utilized, the eggs, attached by the pedicel to a root or stone above, swinging loose. This brooding by the adults doubtless serves the double purpose of providing moisture and securing protection from insects.

Several attempts were made to transfer a parent with a cluster of eggs to the laboratory, that the development and hatching might be observed. Damp moss was tried but it invariably moulded. A small jar kept moist with a wad of water-soaked cotton proved no better. Then, because the air of the laboratory seemed so disastrous, it was suggested opening the bottle, in which the eggs were brought from the field, upside down into an overturned sterile jar. Within the jar, the eggs were placed on a strip of filter paper that was so placed on a piece of glass



From left to right: four embryos from the same cluster of eggs, one removed from the egg, one hatched about twelve hours, one about six hours and one about twenty-four hours. (Magnified three and one half times.) (Photographed by the author.)

above a dish of water that it was constantly wet. No mould appeared, the moisture was constant, and the eggs developed.

An embryo just out of the egg is 2 cm. long; the gills are present but not quite so large as they are a day or two earlier. The head seems larger compared with the rest of the body, than in the adult stage. There is a large quantity of yolk visible which persists for several days.

In this cluster of eight eggs, five were allowed to hatch normally, three were opened for pictures. The hatching of the five covered a period of twenty-four hours. The actual process of hatching was not observed.

There is a small amount of jelly and membrane left; this was noted both in the laboratory and in the field where nests were under observation. Beneath one stone, the pedicel and gelatinous substance was observed after the eggs had hatched. It had turned dark and was tough and leathery.

The embryos just out are very active and show several characteristics of the species. Salamanders, less than a day old, avoid water, wriggle if annoyed and cling to the surface of the glass jar.

The gills shrivel rapidly; at the end of twenty-four hours, there are but mere stubs.

SUMMARY.

In summing up the results of the observations reported above, the writer makes the following statements:

1. The red-backed salamander is found throughout all the eastern United States and Canada.

2. It lives beneath stones and logs, ranging from rather dry to wet places.

3. It is a small salamander, usually about three and one half inches in length.

4. It is almost entirely nocturnal. It is quick of motion and a climber; it cannot live in water.

5. The food consists mostly of live insects, larvæ, worms and spiders.

6. Snakes and frogs are probably its greatest enemies.

7. The protective devices are autotomy and the secreting of a viscous fluid from the tail.

8. The eggs are laid beneath stones in July and early August. One of the parents, usually the female, broods them. The young hatch with three pairs of gills which shrivel in about a day.

My acknowledgments are gratefully given to Dr. Clifton F. Hodge for suggesting the problem, for his active interest and help during the year, to Miss Louise Gulick for aid in collecting specimens and photographic work, to Mr. William T. M. Forbes for help in identifying insects, and to Dr. Newton Miller for his excellent and painstaking work in photography. I aslo wish to thank the many people, who by their interest, advice and criticism have aided in the work.

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ON THE DISTRIBUTION AND MODE OF OCCURRENCE IN THE UNITED STATES AND CANADA OF CLINOSTOMUM MARGINATUM, A TREMATODE PARASITIC IN FISH, FROGS AND BIRDS.

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Contents.

1. Geographical Distribution of *Clinostomum marginatum* 350
2. On the Cysts in the Black Bass 354
3. Behavior of Worms Liberated from Bass-cysts 357
4. On the Cysts in the Frog 360
5. On the Occurrence of the Worm in the Heron 362

1. Geographical Distribution of *Clinostomum marginatum*.

The fluke referred to in this paper was first noticed in this country in 1856 by Joseph Leidy, in the intestine of the pike of the Delaware and in cysts attached to the gills of the sun-fish (*Eupomotis vulgaris*) near the city of Philadelphia. Leidy applied the name *Clinostomum gracile* to it. This generic name after many years of non-use has come into current usage since the late revision of the Trematodes which has received such impetus from the work of Looss ('00) who recognizes this name and Braun ('00) who in a revision of the genus in 1900 recognizes eight species of the genus *Clinostomum*, among them *C. marginatum*. In 1879 R. Ramsey Wright found cysts attached to the gills, branchiostegal membranes and pectoral fins of the yellow perch (*Perca flavescens*) at Toronto, Ontario, which he recognized as being identical with the *Clinostomum gracile* of Leidy and called *Distomum gracile*. His observation extended the geographical range of the species to the St. Lawrence River system. In the same paper Professor Wright reported the finding of *D. gracile* in the bittern (*Botaurus minor*) a fish-eating bird which was the first information as to the definitive host of the worm. Looss in 1885 published an account of the structure of a fluke which had been found encysted in the muscle of a silurid fish from Porto Rico. He gave to this fluke the name *Distomum reticulatum* in allusion to the very peculiar excretory collecting apparatus. Subsequent writers, including Looss himself (Looss '99), have referred this worm to the species *Clinostomum marginatum*, and while there are certain points in which it differs from *C. marginatum* we may for the present so recognize it. This observation however takes the fluke a long way out of the region in which we otherwise know the animal and seriously embarrasses the attempt to deal with the geographical range of the species. In 1888 Leidy reported a fluke from the striped bass (*Roccus lineatus*) which he designated *Distomum galactosomum*. His account of the organization of this form touches on the form of the oral end of the body and the network of collecting vessels in the body-wall, two features of such peculiarity that we cannot doubt but that the subject of his study was none other than *Clinostomum marginatum*, which he had described in 1856 and evidently forgotten. His account located the worm in a marine host for the first time, unless Looss' silurid was marine.

In 1895 MacCallum ('99), of the University of Toronto, found a trematode encysted in the muscles of the frog which he regarded as identical with the ones which Wright had found in the fish and heron and referred to the *D. gracile* of Leidy. In his paper of 1899 MacCallum calls the worm *C. heterostomum*.

His description, while differing in some respects from my material, leaves no doubt but that our material is identical. I shall adopt the name *C. marginatum* in place of the name used by MacCallum following the lead of Braun ('00) in his revision of the group. He also found the same species in the throat of *Ardea herodias* at Danville, Ontario.

In 1898 Linton reported and figured this species under the name of *D. gracile* from the sun-fishes (*Eupomotis pallidus* and *Chænobryttus gulosus*) of Kansas City, Mo. This observation extended the range of the worm to a new river system, the Missisippi and gave us the most western point of its distribution. In 1900 specimens of this fluke were found by myself at Nebish, Mich., encysted in the muscular tissues of the small-mouthed black-bass (*Micropterus dolomieu*), also, though less frequently, in the muscles of the yellow perch (*Perca flavescens*). They were also found later in the same year in the throat of the little blue heron (*Ardea herodias*). Nebish is a small camping place situated on the St. Mary's River, about twenty-five miles below the outlet of Lake Superior. A new locality was thus added to the distribution of the worm though one in the same river system as the Canadian ones.

In 1903 I received, through the kindness of Professor Linton, some pieces of the small-mouthed black bass infected with this parasite. The fish had been taken by the Rev. J. H. Young at Troy, Ohio, on the Miami River, a tributary of the Ohio, and part of the Mississippi system. Some of the worms were encysted in muscle, but others were located in the skin on the internal aspect of the branchiostegal membranes. Their location is shown in Fig. 1 of this article.

In the same year my attention was called by Dr. W. S. Nickeson, of the University of Minnesota, to certain flukes which he had found in frogs, and they were at once recognized as specimens of this species. At about the same time I began to notice them in the frogs of this region brought to the laboratory for use as biological material. The details of the occurrence of the fluke in this region is still under investigation in connection with a study of the parasites of the frogs of this vicinity, so that a fuller account of this part of the subject is reserved for a later occasion. It is important, however, to place on record the occurrence of the fluke in this region, as it extends the range of the parasite considerably beyond formerly recorded limits.

The latest appearance of this form in literature is the record of its recognition in the yellow perch of the Montreal markets by Stafford in 1904, where it is given Leidy's designation *Clinostomum gracile*.

The foregoing facts show that this species is very widely distributed in this country, having been recognized at Kansas City on the West and at St. Paul, Michigan, and as far east as Montreal. It has been seen as far north as St. Paul and as far south as Philadelphia and in Ohio in the center of this territory. The occurrence at Porto Rico is anomalous and cannot be considered until more information is forthcoming. In view of the wide distribution and large size of this species, it seems strange that we are not in possession of a particle of information regarding the early stages of its life-history. The worm is fully developed when it is encountered in the fish or frog and all that is needed for its complete maturity is that the vitellaria shall become active, which they do as soon as the parasite reaches the heron. The details of structure of the worms of the bass have been determined both in total preparations and in serial sections. In the smallest individuals the entire organization is developed. It follows from this fact that there are three hosts involved in the life-history of this species of which the fish or frog is the second and the heron the final one, the first being unknown. The rôle of the fish or frog does not appear to be one of importance from the view point of the ontogeny, but seems merely to be for the purpose of getting the worm to the heron. It thus would appear that the primary host is not one which serves as food to the heron but does serve as food for the bass. This would fit the case of insects as well as any group of invertebrates.

In view of the fact that this parasite infects the edible portion of such important game and food fishes a knowledge of its life history is particularly desirable. While the parasite is fortunately one which is not injurious to the human system, at the same time its presence in the bass and perch unfit them for the table. It is clear that the worm is already widely distributed and is a menace to fish-culture, which at any time may become of great importance. It seems likely that if one were able to take up the problem of this life-history under favorable conditions for instance at some small body of water where the infected fish were abundant and to follow it up at various seasons the missing data could be obtained.

The data given in the foregoing paragraphs are summarized in the following table.

Date.	Observer.	Name Used.	Host.	Seat of Infection.	Locality.
1809	Rudolphi.	*Distomum marginatum.*			
1856	Leidy.	*Clinostomum gracile.*	*Esox.*	intestine.	Philadelphia.
1879	Wright.	*Distomum gracile.*	*Perca flavescens.*	branchiostegal membranes.	Toronto, Ont.
1879	Wright.	*Distomum heterostomum.*	*Botaurus minor.*	mouth.	Toronto, Ont.
1885	Looss	*Distomumi reticulatum.*	Silurid fish.	muscle encysted.	Porto Rico.
1888	Leidy.	*Distomum galactosomum.*	*Roccus lineatus*	—	Philadelphia.
1895	MacCallum.	*Distomum gracile.*	Frog.	muscle.	Toronto, Ont.
1897	MacCallum.	*Distomum heterostomum.*	*Ardea herodias.*	mouth.	Toronto, Ont.
1898	Linton	*Distomum gracile.*	*Eupomotis pallidus.*	pectoral fin.	Kansas City, Mo.
1898	Linton.	*Distomum gracile.*	*Chænobryttus gulosus*	roof of mouth.	Kansas City, Mo.
1901	Osborn.	*Clinostomum marginatum.*	*Micropterus dolomieu.*	muscle.	Nebish, Mich.
1901	Osborn.	*Clinostomum marginatum.*	*Perca flavescens.*	muscle.	Nebish, Mich.
1903	Osborn.	*Clinostomum marginatum.*	*Rana virescens.*	cœlom wall.	St. Paul, Mn.
1903	Young,	*Clinostomum marginatum.*	*Micropterus dolomieu.*	branchiostegal membranes.	Troy, Ohio.
1903	Young.	*Clinostomum marginatum.*	*Micropterus dolomieu.*	muscle.	
1904	Stafford.	*Clinostomum gracile.*	*Perca flavescens.*	gills.	Montreal, Ont.

2. Mode of Occurrence in the Black Bass and Perch, at Nebish, Mich.

The observations now to be described were made at Nebish in 1901 and repeated the following year. Nebish is a small settlement on the American side of the St. Mary's River about midway between Lake Superior and Lake Huron. At that time bass and perch were fairly numerous, beginning in the early part of September and the last of August. As they were not caught much earlier it was the local opinion that the fish migrate to the grounds at Nebish from elsewhere, and this is borne out by the fact that of late years, as a consequence of the work done in improving the channel of the river for navigation, the bass have ceased to come in the fall and the "fishing" is destroyed. I examined as many of the various kinds of fish and invertebrates as possible during my short stay at Nebish in hope of getting some information which would lead up to the discovery of the first host. The only forms on which I recognized the parasites were the bass and the perch. Other fishes which were investigated with wholly negative results were the grass-pike, which is very often caught there, and the sun fish, *Eupomotis*—also very common. Various gasteropods and insects were examined but without any result. I have thus far not been able to obtain any clue to the source of infection of the fish.

The percentage of infected individuals found among the bass was very great. I do not happen to have any statistical data bearing on this point, but the parasite was found in nearly every bass submitted to examination, while in the perch it was much more rare. The cysts were very easily seen, being large, opaque and very creamy white, in marked contrast with the darker semi-translucent muscular tissue in which they lie imbedded. When the fish were skinned in preparing them for cooking the cysts walls were often torn open and the conspicuous worm seen moving on the surface of the meat. The cysts were found in all parts of the lateral muscles, deep and superficial, dorsal and ventral and headwards and tailwards. There was no evidence from their location bearing on the question of the mode of entry which had been adopted by the worms, but the observations of various others who have reported the worm from the gills, roof of mouth, branchiostegal membranes and pectoral fin would indicate that the worm enters the fish in the head region. If so it is difficult to imagine how they reach the places where we find the cysts if they are as large as we find them when they enter. On the other hand, if they enter as small and immature stages we should surely be able to find some evidence of it. Some of the worms should be less fully developed than others, or some of the waste products of the chemical processes involved in growth should be recognizable. So that we are unable to find a solution to this problem with the data at present available.

The number of cysts in single individuals varied greatly. The minimum number found was seven and the maximum number was more than one hundred. The appearance of one of the cysts in situ in the muscle is shown in Fig. 2. They are oval or spherical. They are smaller than those reported by Looss ('85) from the fish which he examined, being 1.3–3 mm. long as compared with 3.5–4 mm. in length according to Looss.

The cysts occupy a space in the endomysium among the fibers of the muscle of the host. As we do not possess many detailed accounts of the structure of trematode cysts or their relation to the host tissues, an account of the facts found in this case may be of some interest. The following methods were employed. The cyst was carefully separated by teasing away the surrounding muscle fibers of a piece which had been fixed and hardened in suitable reagents. The cyst was then cut open and the enclosed worm removed, after which pieces of the wall were submitted to the action of various staining reagents and mounted for microscopical study. In other cases the muscle and enclosed cyst was sectioned serially. In such a series we have transverse sections of the wall and the surrounding muscle tissue, and at the ends of the series there are tangential sections which can be compared with the flat preparation just mentioned. Figs. 3 and 4 are low- and highpower views of such sections, and they show the structural factors involved. The muscle fibers are bent around the cyst as seen on the sides of the section; those in the center which seem to end abruptly at the surface of the cyst do not in reality do so, but are cut off as they run out of the plane of the section. The worm wholly fills the cavity of the cyst, the very small space which is seen in the figure being easily accounted for by the shrinkage of the worm. The structure of the cyst is seen in Fig. 4 to be merely a membrane produced by the condensation of fibrous tissue. There are the usual wavy fibers, thinning out as they reach the endomysium, and continuous with the fibers which fill in the spaces between the muscle fibers, and between the fibers the customary flattened connective tissue nuclei. The flat preparations and tangential sections show very clearly that the cyst is supplied with a capillary network derived from the vascular supply of the muscle, and these capillaries and their contained blood corpuscles can be recognized in the section of the wall as shown in Fig. 4. Looss states ('85) that his observations led him to conclude that the cyst is produced by the host and then lined by a second envelope produced by the worm itself. "Darunter befinded sich eine zweite Hulle, die, anscheinend ein erhärtetes Secret, von dem

Wurme um sich erzeugt wurde, und in dieser liegt eingerollt und zusammengeschlagen das Thier selbst." As to this inner lining found by Looss no traces of it are found in my material, and the cyst is wholly a product of activities on the part of the host tissues. The cyst may be looked upon as a defense made by the host against the parasite. It would be interesting to know the nature of the stimuli which have caused this reaction on the part of the supporting tissues of the muscle whether they are merely mechanical and acting in the form of pressure or whether they are chemical or both. The excessive formation of connective tissue and the growth of a definite and extensive capillary network have resulted from the presence of the worm. In the somewhat analogous case of the cavities produced in oak leaves by the sting of certain hymenoptera for the purpose of housing their larvæ, the normal growth of the leaf is turned aside sufficiently to produce cavities for the larva, walled with a material produced by the leaf from its own substance. In that case the stimulus would seem to be chemical. Whether it is so in this case cannot be determined until we are in possession of more facts connected with the introduction of the parasite to the tissue of the fish.

In the bass, as already stated, the worm entirely fills the cavity of the cyst. In order to determine its position within, several fixed and hardened cysts were carefully dissected out of their place in the muscle and the wall carefully removed under a dissecting microscope. The worms thus found bent and cramped within were macerated for an hour or less in tepid water till they became flexible and could be uncoiled as shown on Fig. 5. They were thus found to be bent twice, both times with the ventral surface outward, first on the level of the meeting of the first and second body thirds, and again on the level of the second and third body thirds. The first of the bends is in the median plane of the body and brings the dorsal surfaces in contact; the ventral sucker is thus exposed and serves to identify the surface posiively. In making the second bend the body does not bend dorsally at once but first there is a twist which brings the dorsal side over the edges of the first and second thirds of the body and then the dorsal surface of the last body third laps over these edges. The worms and cysts in the frog are very different from this. They will receive attention later.

3. Behavior of Worms Liberated from the Bass Cyst.

The living worms are easily liberated artificially by cutting into the cyst wall with a sharp instrument. As soon as a small opening has been made the worm begins to extrude through it almost as if it had been confined under some degree of pressure. It continues to make active movements of extension and contraction which soon result in freeing it from the cyst. It continues to move actively after gaining its freedom. In the course of nature the worm on emerging from the fish cyst would find itself in the stomach or intestine of a fish-eating bird; those which were removed artificially were received and kept in shallow vessels of fresh water where their activities could be watched. The surroundings in which they thus found themselves were quite unnatural and perhaps it was for this reason that they were so very active. The movements did not result in locomotion, though they produced constant changes in the form of the body, but the worm did not progress. Some trematodes adhere very forcibly by their suckers to any object with which they come in contact but there was no attempt made on the part of these to use the suckers for that purpose. Some of the various movements were so irregular and indefinite as to preclude the possibility of grouping them under any general head, but there were others which were definite and constant enough to be classed under either of two groups which I have called "poses" from the fact that in each case a series of movements took place culminating in a certain bodily form or attitude which was only momentarily maintained and after which the body relaxed and fell back to its ordinary resting posture. These two poses were seen often enough to justify the conviction that they are a constant feature of the behavior of the worm and so merit a detailed description. The two poses will be designated the "suctorial pose" and the "swimming pose." Sometimes the same pose is repeated several times in succession, at other times the two alternate irregularly.

The suctorial pose is represented in Fig. 6 as seen in the living animal and in Fig. 7, a view of a sagittal section of the anterior end of the body of an animal which happened to be caught in this pose at the moment of fixation. The region of the body chiefly active in the production of this pose is the part anterior to the ventral sucker. Ordinarily this part of the body has the shape of an obliquely truncated cylinder. In assuming this pose the worm draws the end of the body back into the interior, the side walls being thicker and acting as the rim of a sucker projecting considerably beyond the level of the center. This inversion of the oral end of the body is brought about by the contraction of fibres of the parenchyma muscle system which run longitudinally in the body. Some of these fibres are shown in Fig. 7. Their contraction pulls the center of the oral end back and the margin is left projecting. If this action were to take place at a moment when the oral end was in contact with a soft surface, for example, such a surface as the mucous coat of the stomach or throat of the heron, then the soft substance of the host would be sucked into the cavity of this sucker and a powerful adhesion of the parasite to the host would result. A worm liberated naturally from the cyst would meet conditions which would furnish responses to this movement, so that instead of being merely a momentary pose it would be useful and so continued. On the other hand, in the case of the artificially liberated worm such stimuli being absent the worm returns to its customary form. The sucker thus formed is additional to the two usual trematode suckers. We may suppose that in as large a parasite as *Clinostomum* and one which lives in such an exposed place as the gullet where large masses of food are pressed against it additional adhesion would be needed to protect it against being dislodged by the pressure of the food as it is being swallowed. This striking movement had thus evidently a purposive character, as can be seen if we take into account the environment for which it has been developed.

The "swimming pose" is shown in Fig. 9. In this case the body is made broader and flatter than usual, the ventral surface becoming somewhat concave. The margins of the posterior part of the body are reduced to fine sharp lines like fins which do not extend into the front part of the animal but crossing it ventrally converge toward the ventral sucker. The line shown in the figure is not visible in any specimens of the worm after preservation and is only a momentary structure. The flattened form of this pose too is only momentary, and no preserved specimens show it. In all cross sections of the worm the body is elliptical and has a thickness of nearly half its breadth.

The transverse parenchyma muscles are the ones which are used in the swimming pose, the longitudinal muslces being at rest. Alternate contractions of the transverse and longitudinal muscles would throw the animal first into one, then into the other of these positions. The observation of certain leeches furnished the suggestion for calling this the swimming pose. In the leeches the margins of the body are thinned in this way and reduced to a thin lateral fin and the worms swim rapidly through the water. In the specimens of *Clinistomum* which were under observation no vibrations of the body were made and the pose was not turned to any account. It is possible that in nature this body form would be adapted to progression through the chyle and chyme of the bird and would be the means by which the worm reaches the throat toward which it is travelling from the stomach or intestine. It would be very interesting to experiment with these worms by removing them from cysts and placing them on surfaces as much like the avian mucous membranes as possible so as to ascertain whether these poses are as adaptive as they seem to be.

4. On the Cysts in the Frog.

The first mention of the frog as a host of *Clinostomum* is by MacCallum ('99), who reports it from the pectoral muscles of frogs found in Ontario. We have been finding it now for several years in the frogs caught in the vicinity of St. Paul which are used in our biological courses.[1] The mode of infection of the frog is different from that of the bass. The cysts, instead of being located in the muscular tissue, are seldom found there, but are in the peritoneal lining of the cœlom or in the lymph spaces between the skin and the muscles of various parts. Text-figure 1 is drawn from the most considerably infected specimen which I have met. The cysts are located in the cœlomic cavity;

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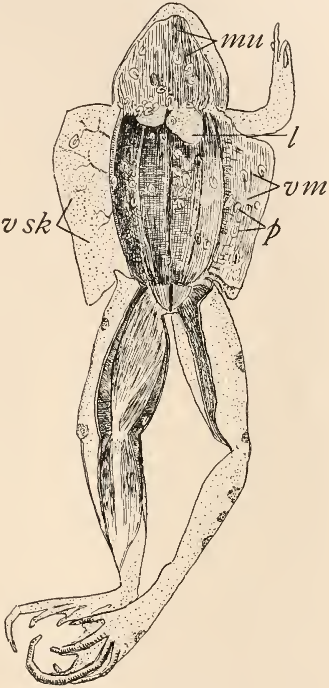
[1] It is most probable that frogs and fishes in many parts of this country are infected by this parasite. Any information as to localities elsewhere where it has been noticed would be very gratefully received by the writer. A study of the relation of the worm to the frog is now in progress, and it is hoped that clues to the early history of the worm may be obtained through its connection with the frog.

especially in the region dorsal to the heart. They are found here most frequently. They are also found in the ventral wall of the cœlomic cavity, in the floor of the mouth between the muscles and the skin and even in the leg between the muscle and the skin. In no case have the cysts been found in the frogs of this region in the interior of muscular tissue surrounded by the fibers as they are so characteristically found in the fish. I have as yet not been able to find any reason for this difference in the location of the cysts with regard to the host, but the position of the worm within the cyst is considerably affected, as will be explained fully in a moment.

The ratio of infection of the frog does not appear to be as high as it is in the fishes studied at Nebish. The percentage of frogs not infected is very high, indeed a large number can be gone through often without any cysts being found. The number of cysts in a single individual is usually not large; the frog figured is quite exceptional in that respect. Data for an exact statement on these points are not at present worked out.



View of ventral surface after the removal of the cœlomic viscera, showing the numerous encysted flukes in place. *l*, lung; *mu*, muscles of floor of mouth; *p*, flukes in place; *v*, ventral muscles of cœlomic cavity; *v sk*, skin of ventral cœlomic wall. ×2/3 natural size.

The relation of the worm to the cyst and to the underlying muscle of the body wall is shown in Fig. 9. The muscle fibers run along below the cyst, their course entirely uninfluenced by the presence of the cyst. A comparison of Fig. 9 with Fig. 2 shows the difference of habit in this respect at a glance. The preparation from which Fig. 9 was drawn shows five cysts in an area half an inch wide by a quarter of an inch across. It is removed from the right half of the body wall of the frog shown on page 361. The whole piece was stained and mounted in balsam and one of the cysts drawn in Fig. 9. The cyst is much larger than the worm and there are spaces within unoccupied by the worm. The worm in most cases is bent once, only the ventral surface being turned outward. In some cases the bend is not in the center, and then the longer end may be bent slightly as in the one shown in Fig. 9. The frog cysts show the presence of a rich network of capillaries spread out over their surface. These are derived from vessels which come from large and conspicuous vessels running in the space between the peritoneum and the muscle layer. The ordinary surface of the peritoneum does not possess these capillaries, which are evidently a growth developed as a result of the presence of the cysts.

The specimens of *Clinostomum* seen in the frogs are all virtually fully matured, having the full size of the heron specimens. Their inner organization too is identical in appearance with that of specimens from the fish, and excepting as to the vitellaria with that of the heron. There is thus no room for an hypothesis as to the frog being the first host and the medium by which the fish is reached, which hypothesis we might be tempted to frame, knowing that the frog is one of the foods of the bass, and other predaceous fishes. We are thus left to suppose a first host, possibly an invertebrate, followed by a second intermediate host, the fish which serves as a medium of transmission from the unknown first host to the heron. The case of a second host which serves to transmit a parasite from a first host where it develops to a final host where it matures and where sexual reproduction takes place has been recognized for a number of trematodes. A list containing 28 species is given by Braun (93, pp. 864–866), in which this species is not included. In some of these the frog or one of the fishes serves as the medium by which the final host is reached.

5. On the Occurrence of the Worm in the Heron.

A single specimen of the heron sent me by express from Nebish in October happened to be infected with this parasite and furnishes all that I have myself observed on this point. The bird had been shot and had been dead a day or two before it came into my possession. The worms were still alive and suitable for fixation and preservation for histological study. The worms were found in considerable numbers adhering to the wall of the throat by means of the anterior end used as a sucker in the manner referred to above. On being removed they were found to be mobile but no data as to their movements were recorded. The stomach and intestine of the heron were carefully examined to ascertain whether those organs were infected or not. No worms were found there. The stomach however contained the remains of the bodies of five fishes in various stages of digestion all of which were too decomposed for recognition with entire certainty but their elongate shape, their size and the fauna of the region made it virtually certain that they were sepcimens of the yellow perch. This observation furnishes the evidence of a connection between the fish and the final host. The liberation of the worms in the stomach and their migration of the worms in the stomach and their migration and adhesion to the wall of the throat is of course the first event of their life in the avian host followed by the production of eggs and their passage to the uterus where we find them in great numbers in the specimens taken from the heron. It is not exactly clear why the eggs are not passed directly from the body of the heron after the manner general in the flukes. Here however there is a large uterus into which the eggs pass and where they accumulate, for reasons as yet unknown. The eggs when they are expelled from the fluke must pass down the alimentary canal of the heron and fall with the feces in the water or less probably on the ground, where they make their way to the first host. The eggs in the uterus show no signs of development so that the ontogeny remains for the present a wholly unknown quantity.

Summary.

1. The first host and early life-history of *C. marginatum* are entirely unknown.

2. It passes a period of quiescence encysted in the muscle tissue of various predacious food fishes and in the lymph-spaces of frogs, in various localities ranging from Missouri through Ohio to Pennsylvania, and north as far as Minnesota, Michigan and Ontario, Canada.

3. It finally reaches an avian host which is some fish-eating bird, such as the bittern or the heron.

4. The cyst in the fish is a connective tissue structure produced from the endomysium of the host with a special vascular equipment.

5. The living worms on being artificially liberated from the cysts perform characteristic movements adapted to finding locations in the final host.

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Explanation of Plate I.

Fig. 1. View of the inner aspect of the right half of the lower jaw of a specimen of the small-mouthed black bass, from Ohio; showing the white cysts of *C. marginatum* at the bases of the branchiostegal rays, and black spots of an unidentified nature. Natural size.

Fig. 2. A cyst of *C. marginatum* in place among the muscle fibers of the bass from Nebish, Mich., from a glycerine preparation after teasing off part of the muscular tissue.

Fig. 3. View of a section passing through a cyst in place in the muscle tissue of the bass, from Nebish, Mich. Camera lucida, ×24 diameters.

Fig. 4. View from a section of a cyst in a plane transverse to the muscle tissue, showing in detail the histological structure of the wall of the cyst; from bass from Nebish, Mich. Camera lucida. ×340 diameters.

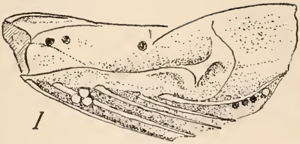
Fig. 5. Drawn from a specimen of *C. marginatum*, which had been removed from the cyst after fixation in alcohol and relaxed by slight maceration; from bass from Nebish, Mich.

Fig. 6. Sketch of living animal in "suctorial pose" as seen from ventral side; from bass, Nebish, Mich.

Fig. 7. From a sagittal section of anterior end of worm which died in "suctorial pose," from heron, Nebish, Mich. Camera lucida, × 38 diameters.

Fig. 8. View of ventral surface of a living animal in the "swimming pose," from bass, Nebish, Mich.

Fig. 9. View of a portion of the muscles of the ventral cœlomic wall of frog, showing a cyst and enclosed worm. From specimen after clearing and mounting in balsam. St. Paul, Minn. Camera lucida, × 35 diameters.



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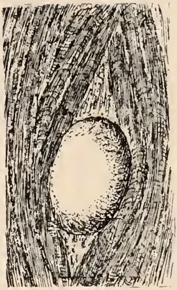

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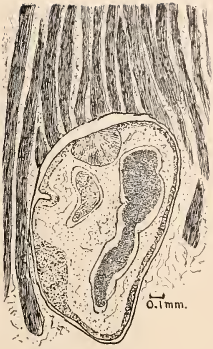

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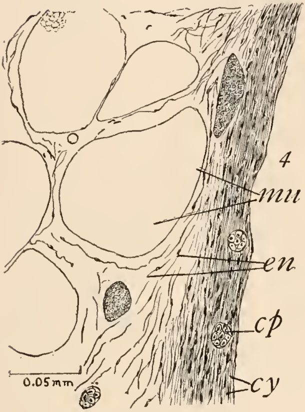

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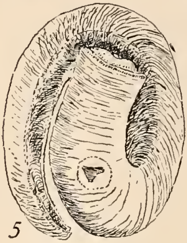

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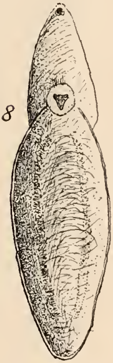

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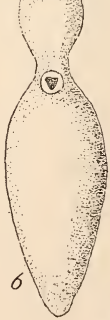

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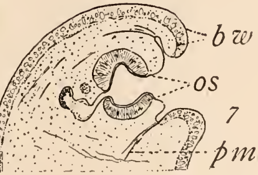

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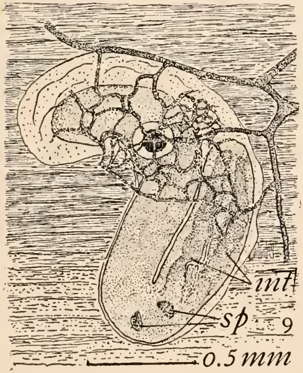

9

HENRY LESLIE OSBORN

THE SCALES OF FRESHWATER FISHES.

T. D. A. COCKERELL,

University of Colorado.

Professor N. S. Shaler,[1] writing of his experiences as a student under Agassiz, said: "I acquired a considerable knowledge of the literature of ichthyology, becoming especially interested in the sytem of classification, then most imperfect. I tried to follow Agassiz's scheme of division into the order of ctenoids and ganoids, with the result that I found one of my species of side-swimmers had cycloid scales on one side, and ctenoid on the other. This not only shocked my sense of the value of classification in a way that permitted of no full recovery of my original respect for the process, but for a time shook my confidence in my master's knowledge."

I quote this, because the breakdown of Agassiz's original system of classification by scales affected not only Professor Shaler, but many others, to the detriment of lepidology. Dr. Jordan, in his great work, "A Guide to the Study of Fishes" (1905), devotes only two pages to the discussion of scales, with a couple of figures. In the descriptions of fishes published by various authors, much is made of the number and size of the scales, but little of their structure, and it rarely happens that an ichthyologist even takes the trouble to remove a scale from the fish he is studying, in order to determine its characters. These remarks apply especially to the teleosts—the ordinary fishes of modern times, and it is of course true that students of the "ganoids" and their allies, mostly fossil, have never neglected the scales. The ganoids have greatly thickened scales, usually rhombic in form, which are well preserved in the rocks; as Agassiz so well insisted, they afford a most important aid to the classification of these animals, particularly in the numerous cases in which the skeleton is poorly preserved. Recent researches, while destructive to certain theories which Agassiz considered important, have only emphasized his doctrine that the scale is of great taxonomic value. I have paid little attention to the ganoids and their relatives, and will not attempt to discuss them, here, but may call attention to the important memoir by Goodrich[1] in which it is shown that the so-called ganoid scales can be divided into three very distinct groups, called the cosmoid, palæoniscoid ganoid and lepidosteoid ganoid. Mr. Goodrich, having distinctly differentiated these types, applies his work to the classification of some difficult forms, with striking results.

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[1] *Atlantic Monthly*, February, 1909, p. 222.

Teleostean scales, the ordinary imbricated readily removable scales of fishes, were classified by Agassiz as cycloid and ctenoid, and his names are still in current use. The cycloid scale is one in which the apical margin is what a botanist would call entire, that is without teeth or serrations. The ctenoid or comb-like scale has the margin dentate, serrate or spiny. It is now well-known that these are not fundamental divisions, some families having both ctenoid and cycloid types, and as Shaler has stated, it is possible for both kinds to exist on a single flat-fish. Nevertheless, the distinction is usually an important one, while the scales have innumerable other characters of value, not used in Agassiz's sytem of classification.

My own work with fish-scales had what might be called an accidental beginning. In the course of class-work, my students and I took occasion to examine the scales of the fresh-water fishes of Colorado. We soon perceived that they had excellent distinctive characters, and thereupon sought literature on the subject. Failing to find anything satisfactory, we wrote to Dr. D. S. Jordan, who promptly gave us the desired information, or rather, indicated the lack of it. Girard, in his report on the fishes of the Mexican Boundary Survey, had given numerous figures of the scales of fresh-water fishes, but had not discussed or classified them. About fifty years ago it appears that it was seriously intended to work up the lepidology of the American teleosts, but for some reason nothing came of it, beyond the publication of the figures mentioned. Later, Dr. Boulenger gave me access to the work of Fatio (1882), in which the scales of all the fishes of Switzerland are figured; also to the great work of

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[1] *Proc. Zool. Soc. London*, 1907, pp. 751–774.

Sauvage on the fishes of Madagascar, containing numerous figures of scales of Acanthopterygian fishes. In other writings, here and there, are various figures of scales, usually with little discussion. The scales of *Gadus* (codfish and allies) have been beautifully figured and fully discussed by Dr. H. W. M. Tims (1905).[1]

Dr. Jordan, with great kindness, sent me a fine series of freshwater fishes from the collections of Stanford University, while Dr. Evermann was equally good in supplying numerous species from the Bureau of Fisheries. I thus obtained nearly all of the principal forms of North American Cyprinidæ or carp-like fishes. The following summer I visited the British Museum, and was indebted to Dr. Boulenger for the opportunity of investigating the scales of nearly all the principal old-world cyprinids, and a like series of African characinids. I also obtained through him many African fishes of other families. Quite recently I have received from Dr. Eigenmann, of the University of Indiana, an extremely fine series of South American characinids. With all these, and some others, it has been possible to test rather thoroughly the value of scale-characters, and the result has been to show that while they are not rarely deceptive, through convergence, they are on the whole of great taxonomic importance. As in most other taxonomic work, there are disturbing elements due to individual variability and differences of age, but while these are sure to lead to various minor errors, they will not much affect the broader results. The key to the origin of the sculpture of a teleostean scale is apparently to be found in that ancient type the bowfin of North America, *Amia calva*. Fig. 1 shows part of the base of a scale of this fish, which it will be observed consists of longitudinal strands or fibres, separable elements which fray out basally.[2] In the apical field these are directed toward a broad rough nuclear area. A close approximation to this is found in a very old type of teleosteans, the lady-fish, *Albula*. In this, however, appear also the beginnings of the radial lines, extending from the nucleus of the scale to the margin. As we go higher in the scale of fish-evolution, these radiating lines or *radii* often become very prominent, while the longitudinal strands usually become united above and below, forming circular fibers which we have designated *circuli*. The nomenclature of these structures was based on a normal highly-developed scale, in which the circuli deserved their name, and since then the term has been applied to the same elements wherever found, so that I have had to refer, rather illogically, to *longitudinal circuli*. Perhaps it would be better to call them fibrillæ.

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[1] Dr. B. L. Chaudhuri informs me that Dr. John McClelland published an account of the scales of Indian Cyprinidæ in the appendix to his work on these fishes, in 1839. This I have not seen.

[2] See Smithsonian Misc. Coll., Vol. 56, No. 3, p. 2, 1910.

On looking at a normal scale, in which the circuli are strictly circular, it would be natural to regard them as lines of growth, like those on a snail's shell. There are real lines of growth, however, and these do not necessarily coincide with or have anything to do with the circuli.

In the salmon and trout (*Salmo*) the scales are strictly cycloid, and have only circuli. Fig. 2 shows a scale of the bluefin, *Argyrosomus nigripinnis*, a member or the salmon family. This shows a marked deviation from the simple *Salmo* type, in that there are distinct laterobasal angles, the circuli of the apical field are less dense than those of the basal, and there are slight indications of apical radii. It is no very great step from this to the scale of *Moxostoma aureolum*, one of the suckers (Fig. 3), but it will be noted that in the sucker, as indeed in all members of the Catostomidæ so far examined, there are very distinct basal radii. In the typical suckers, *Catostomus*, the scale is oval, not unlike that of *Salmo* in shape, and there are radii all around. This is well shown in Fig. 4, *Pantosteus santa-anæ*, from California. The possession of basal radii separates the Catostomidæ from the great majority of American cyprinids, although a few genera of the latter family have them, notably *Chrosomus* (Fig. 5, *C. dakotensis*), the scale of which closely resembles that of *Pantosteus* in sculpture, though differing in shape. Among the old world cyprinids (carp family) basal radii are very common, and it is curious that the common European minnow (*Phoxinus phoxinus*) has scales of quite the same type as the American *Chrosomus*. There is reason to believe that the suckers are an ancient family, close to the old stem from which the carp family arose. They are nearly confined to North America, though sparingly represented in eastern Asia. From this circumstance one might look for the earliest types of Cyprinidæ also in America, but the strong indications are that they are old-world forms, the modern American cyprinids, with a few possible exceptions, having arrived in comparatively recent times from Asia. Nevertheless, the carp family in this country must be only of comparatively, not actually, recent origin, since it has had time to develop innumerable species, and a considerable series of endemic genera. The distinctness of the American Cyprinid fauna has indeed been emphasized by the scale work, it being shown that the so-called *Leuciscus*, *Rutilus*, etc., of America are not congeneric with the European fishes bearing these names. It is probable, in fact, that all of the American Cyprinidæ are generically distinct from those of Asia and Europe.

Returning again to the bluefin scale, we can take a new point of departure in Fig. 6, the Asiatic cyprinid *Squaliobarbus curriculus*. Here the general form remains the same, except for a moderate elongation, the apical circuli become more widely spaced, the apical radii are evident, but there are no basal radii. A European scale of nearly the same type is that of *Leuciscus illyricus* (Fig. 7). In other cases, the scale may be more parallel-sided, with a broad truncate base, as is shown in the Asiatic *Labeo sladoni* (Fig. 8) and *Rhinogobio typus* (Fig. 9).

In order to illustrate the value of scale characters within a limited group, I give a series of figures of species usually referred to *Leuciscus*, the dace and its allies. *Leuciscus rutilus* (Fig. 10) and *L. friesii* (Fig. 11) are European; *L. hakuensis* (Fig. 12) and *L. jouyi* (Fig. 13) are Japanese. The remaining four are North American, and because of their very different scales, and other reasons, I have removed them to another genus, *Richardsonius* Girard. *R. orcutti* from California (Fig. 14) constitutes a distinct subgenus, the scale having basal radii. *R. pulchellus* (Fig. 15) is of the Rocky Mountains. *R. carletoni* (Fig. 16) is a distinct type from Maine, while *R. thermophilus* (Fig. 17) inhabits warm springs in Oregon. It will be observed that in the European forms the large scales have exceedingly fine circuli. In the American, the scales are very much smaller, and the circuli are very coarse and widely-spaced. This is in general a characteristic distinction between New World and Old World Cyprinids, although some of the Old-World genera have widely-spaced circuli. The two Japanese species, as one might expect, are rather intermediate between the European and American. They probably should constitute a new subgenus or genus. *Leuciscus illyricus* (Fig. 7) has widely spaced apical circuli, and therein departs in the direction of the Japanese and American fishes.

The Characinidæ are a large family of freshwater fishes occurring in the Ethiopian and Neotropical regions, but not elsewhere, with the exception of a few which enter the Palæarctic in the Nile Valley and the Nearctic in southern North America. This curious distribution has naturally aroused interest among naturalists, and while it is probable that America was their original home, it is a question whether they reached Africa across what is now the Atlantic, or once inhabited the north and passed southward into the Ethiopian area. I should think the latter supposition more probable, but for the fact that as yet we have no trace of fossil characinids outside of their present area. The characinids belong to the same general group as the cyprinids, but are unquestionably more primitive, in spite of the fact that many of their members are exceedingly specialized in certain particulars. Among the African characinids is a group, the Alestini, having a very characteristic type of scale.[1] This, which I call the alestiform scale, is more or less hemispherical in outline, cycloid as to the margin, with a few very strong radii. It seems to be an old type, because we find it nearly repeated in certain of the relatively primitive families, as for instance the Phractolæmidæ (*Phractolæmus ansorgii*, Fig. 17*a*), a rare type from the Niger and Congo rivers. The *Phractolæmus* scale, however, is incipiently ctenoid. A different scale, yet of the general alestiform type, is found in *Pantodon buchholzi* (Fig. 18), another rare and isolated genus from the Niger and the Congo. On this fish the circuli have a peculiar and very characteristic bead-like appearance. Passing to the more specialized cyprinids, we find the alestiform scale not uncommon, and a good example from an Asiatic fish (*Barbus mahecola*, Fig. 19) is given. It will also be noted that *Leuciscus rutilus* of Europe (Fig. 10) has much the same sort of scales though lacking the characteristic lateral radii of the Alestini and the *Barbus*.

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[1] See Smiths. Misc. Coll., Vol. 56, No. 1, plate 1, figs. 4, 5, 6.

In some of the cyprinid alestiform scales the nuclear region is broken up into polygonal areas, but in the curious African Mormyridæ[1] there is a characteristic system of anastomosing radii, forming a network. This is unique, so far as material seen by me shows, except for the case of *Heterotis niloticus* (Fig. 20), a member of the ancient and now much reduced family Osteoglossidæ. In this fish, which inhabits tropical Africa north of the Equator, the network is extremely well developed.[2]

An extremely distinct and interesting type of scale is found in the tench of Europe, *Tinca vulgaris* (Fig. 21). This seems to be a relatively old type, formerly more abundant. Through the kindness of Dr. A. S. Woodward, of the British Museum, I was able to examine the miocene fossil species *Tinca furcata* of Agassiz, and the nominal but apparently synonymous *T. magna* of Winkler, and found the scales to be wholly characteristic of the genus. It is a singular thing that in North America there exists a fish called *Algansea tincella*,—named *tincella* by Valenciennes because of its resemblance to a tench,—which has scales of the same general type though not so extreme. Mr. Regan has described two other species of the genus *Algansea*, *A. affinis* and *A. stigmatura*, and he informs me that they have scales like those of *tincella*.

On the other hand, there are scales which so far as shape goes resemble those of *Tinca*, and yet offer marked differences in the details of the sculpture. Such is the scale of the Asiatic *Schizothorax biddulphii* of Gunther (Fig. 22). Among the American cyprinids, especially the smaller forms commonly known as minnows, there are many scales which at first sight look alike. In some cases, there is actually no definable difference, but often sufficient familiarity with the scales enables one to recognize them without difficulty. I illustrate a series of such forms, *Lavinia exilicauda* (Fig. 23), *Phenacobius mirabilis* (Fig. 24), *Notropis galacturus* (Fig. 25) and *Pimephales anuli* (Fig. 26); also a similar European scale, *Gobio fluviatilis* (Fig. 27). In all these scales there is much variation, which is at times confusing, although it usually does not affect the fundamental character of the pattern. Thus I described the scale of *Chondrostoma soëtta*, from Italy, as having few apical radii, distinguishing it thus from certain Spanish species. Noting later that this did not agree well with Fatio's figure, I asked Mr. Regan to look into the matter and he found that on the same fish immediately adjacent scales had many and few radii respectively. Consequently my diagnostic character proves of no value, but it will be noted that the variation affects the development rather than the character of the markings. Greater differences are noted when the scales are taken for different parts of the body, and for purposes of comparison I always take them from near the middle of the side, close to the lateral line. Fig. 28 and 29 show an extreme case, that of the curious little fish *Ericymba buccata*, from Indiana. Fig. 28 is a normal scale, and Fig. 29 is from the subdorsal region about 5 mm. in front of the dorsal fin. These two scales are from the same fish.

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[1] Smiths. Misc. Coll., Vol. 56, No. 3, p. 2, figs. 1, 2.

[2] Since this was written I have determined that all the Osteoglossidæ, and also all living Dipnoans (lung-fishes) have the radial network.

Occasionally very marked abnormalities occur. Thus on a specimen of *Ostebrama feœ* I found a greatly enlarged scale on the middle of the side, below the lateral line. It was about 8.5 mm. long, six times as long as the exposed parts of the normal scales, but not differing essentially in sculpture. The age of the fish is also often of importance. Fig. 30 shows a scale of *Gila robusta* from Arizona. It was taken from a young fish, and for some time I did not know that the adult scales of *Gila* have a characteristic basal lobe, as indeed was figured long ago by Girard in the report of the Mexican Boundary Survey.

There is an interesting case outstanding, in which the value of scale characters for the separation of very closely allied fishes has yet to be determined. From Stanford University I received a number of specimens of *Myloleucus symmetricus*, collected in different localities. These were sent principally because Dr. J. O. Snyder had already noticed the variability of this fish in other than scale characters, and it was suspected that it might include some separable forms. A study of the scales revealed four apparently distinct types. (1) Scale broad, small. Navarro River, Gualala River (California) and north to Drew Creek in

Oregon. (2) Scale broad, large. Napa River (California). (3) Scale oblong, more weakly sculptured. Ukiah Creek; Russian River (California). (4) Scale oblong, strongly sculptured. Conchilla Creek (California).

It appears from this that there is a broad-scaled form to the north coming south as far as Gualala River. From this it is only a few miles to Russian River, where the fish is oblong-scaled. Passing beyond, however, we find another broad-scaled variety in Napa River. It is suggested that the broad-scaled fishes, when in competition with the oblong-scaled, may get the better of them, and that the Napa River fish represents an invasion from the north away from the coast. All this, however, is at present based on far too little evidence, and is given here partly as an example of the kind of problem the scale-work brings up, and partly in the hope that someone will make an exhaustive study of the matter. Up to the present, I have not been able to get any further information, beyond what is given by Dr. Snyder in Bull. Bureau of Fisheries, XXVII, p. 175. In general, Dr. Snyder's results, based on other than scale characters, agree with mine, but he finds no difference between the fishes of Russian and Napa Rivers. No less than five specific names have been given to *M. symmetricus* as now understood; probably two or three of these will be available for the necessary segregates. While most fish scales fall readily into a systematic arrangement, we occasionally meet with one which differs greatly from any other known to us. Such is the scale of *Kneria cameronensis* Boulenger (Fig. 31), a small fish belonging to a peculiar family found only in tropical Africa. In this the radii run from one end of the scale to the other and the circuli are for the most part broken up into peculiar marks between them.

The study of fish-scales opens up a vast new field, the scales of most of the common fishes being still undescribed. Fish scales also may claim our attention for their beauty as microscopical objects. Their preparation involves no difficulty; they are best mounted dry between thin microscopical slides; or when small, they may be mounted on a slide under a cover glass. At first, I placed them in balsam, but this greatly obscures the markings, and is altogether undesirable.

For the means of obtaining the photographic figures (the work of Mr. T. C. Black) I am indebted to a grant from the American Association for the Advancement of Science. The drawings are by Miss Evelyn V. Moore. In this paper I have described and figured only scales of fresh-water species. At some future time, it may be possible to give a similar account of the marine families, and of several fresh-water groups now omitted.

EXPLANATION OF PLATE I.

FIG. 1. *Amia calva.* Plymouth, Indiana. Dr. Evermann.

FIG. 2. *Argyrosomus nigripinnis.* Dr. Graenicher.

FIG. 3. *Moxostoma aureolum.* Dr. Graenicher.

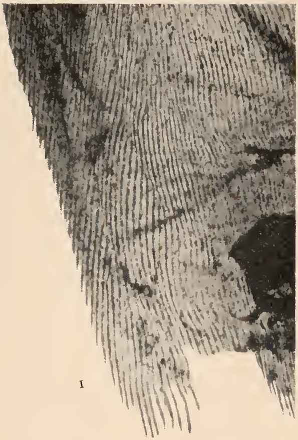
FIG. 4. *Pantosteus santa-anæ.* Santa Ana River, California. Stanford University.

FIG. 5. *Chrosomus dakotensis.* Valentine, Nebraska. Bureau of Fisheries.

FIG. 6. *Squaliobarbus curriculus.* Mountain stream near Kiu-Kiang (Styan). British Museum.

FIG. 7. *Leuciscus illyricus.* R. Tadro, Dalmatia (Dr. Werner). Brit. Museum.

FIG. 8. *Labeo sladoni.* Mandalay (F. Day). Brit. Museum.



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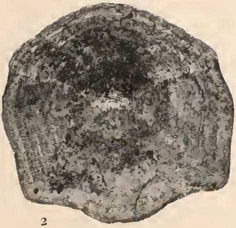

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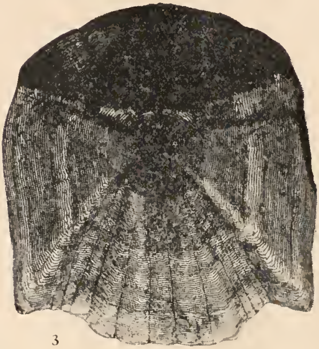

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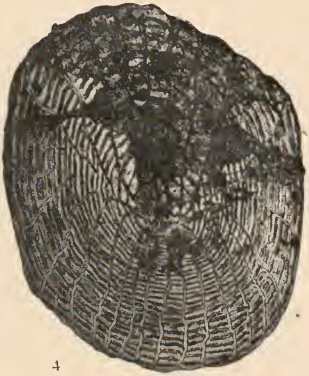

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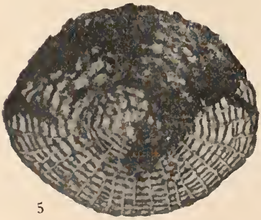

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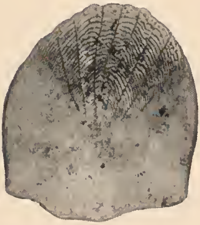

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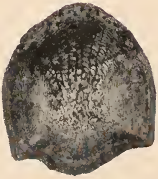

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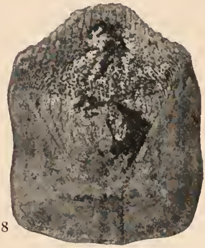

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T. D. A. COCKERELL

EXPLANATION OF PLATE II.

FIG. 9. *Rhinogobio typus.* Kiu-Kiang (Styan). Brit. Museum.

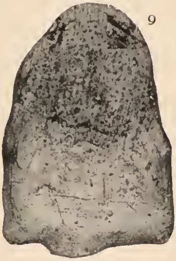
FIG. 10. *Leuciscus rutilus.* Salisbury, England (Odgen Smith). Brit. Museum.

FIG. 11. *Leuciscus friesii* = *L. meidingeri.* Lake of Derkos, Constantinople. Brit. Museum.

FIG. 12. *Leuciscus hakuensis.* Yamada, Japan (R. Gordon Smith). Brit. Museum.

FIG. 13. *Leuciscus jouyi.* Sasuma, Japan (Anderson). Brit. Museum.

FIG. 14. *Richardsonius (Temeculina) orcutti.* Santa Ana River, California. Stanford University.



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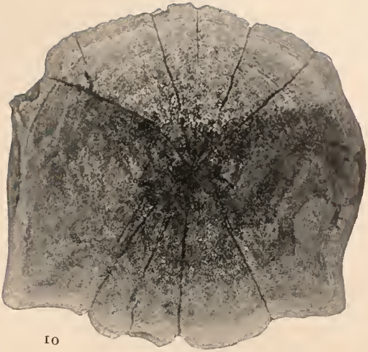

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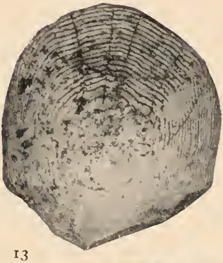

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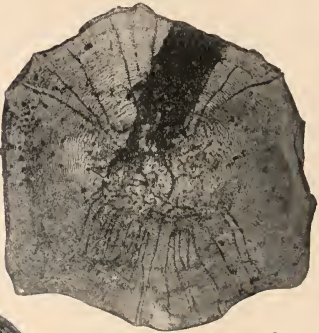

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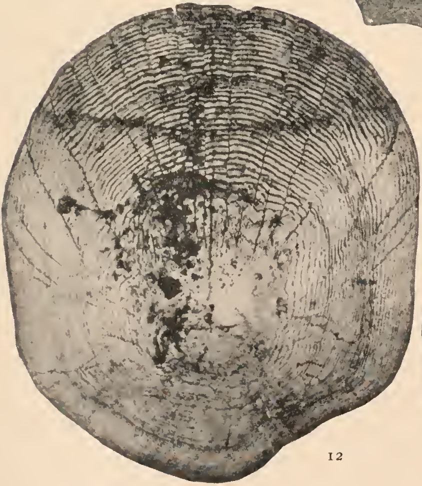

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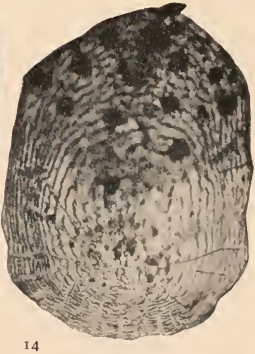

14

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EXPLANATION OF PLATE III.

FIG. 15. *Richardsonius (Tiogma) pulchellus.* Alamosa, Colorado. Stanford University.

FIG. 16. *Richardsonius (Cheonda) carletoni.* Cross Lake Thoroughfare, Maine. Bureau of Fisheries.

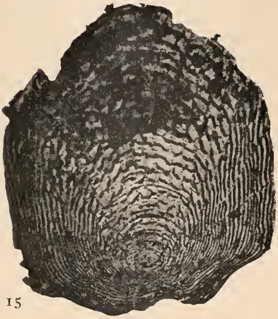
FIG. 17. *Richardsonius thermophilus.* Warm Spring, Harney Co., Oregon. Stanford University.

FIG. 17*a*. *Phractolaemus ansorgii.* Brit. Museum.

FIG. 18. *Pantodon buchholzi.* Brit. Museum.

FIG. 19. *Barbus mahecola.* S. Canara (F. Day). Brit. Museum.

FIG. 20. *Heterotis niloticus.* Brit. Museum.



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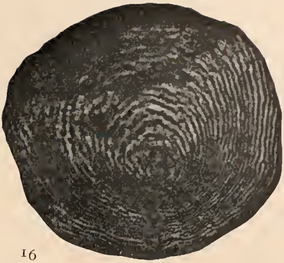

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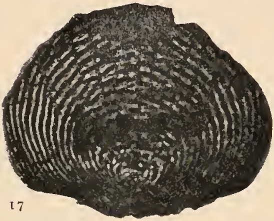

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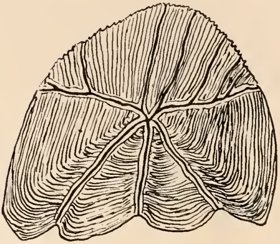

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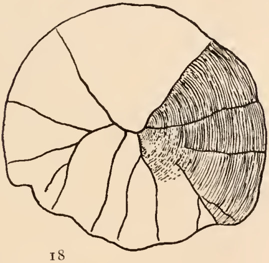

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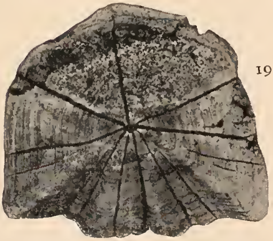

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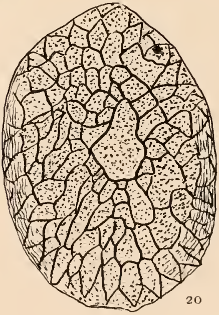

20

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EXPLANATION OF PLATE IV.

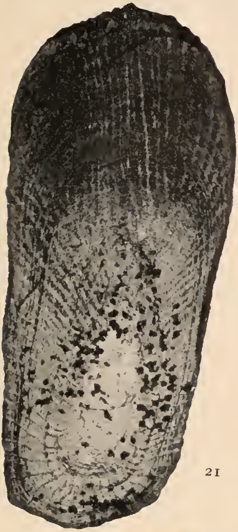
FIG. 21. *Tinca vulgaris.* Constantinople. Brit. Museum.

FIG. 22. *Schizothorax biddulphii.* Lake Balyk-ky near Nijo. (St. Petersburg Mus.) Brit. Museum.

FIG. 23. *Lavinia exilicauda.* Coyote Creek, California. Stanford University.

FIG. 24. *Phenacobius mirabilis.* Bureau of Fisheries.

FIG. 25. *Notropis galacturus.* Saltville, Virginia. Bureau of Fisheries.



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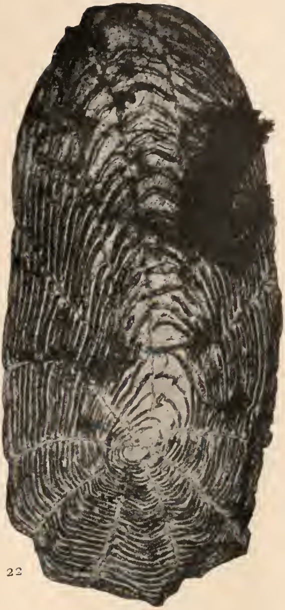

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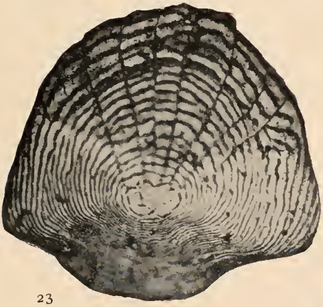

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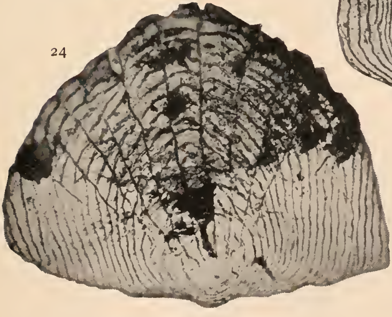

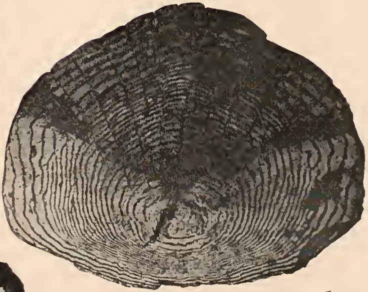

25

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Explanation of Plate V.

Fig. 26. *Pimephales anuli.* Lunkasoos Lake, Maine. Bureau of Fisheries. (Note straight edge of skin across middle of scale.)

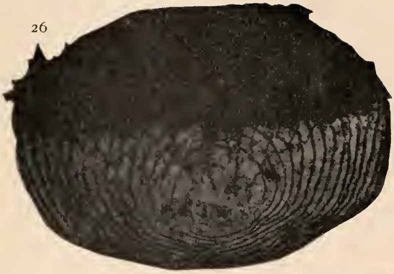
Fig. 27. *Gobio fluviatilis* (*G. vulgaris*). R. Neckar near Canstatt (Stuttgart coll.) Brit. Museum.

Fig. 28. *Ericymba buccata.* Wild Cat Creek, Indiana. Bureau of Fisheries. Figure reversed.

Fig. 29. *Ericymba buccata.* Wild Cat Creek, Indiana. From subdorsal region, in front of dorsal fin.

Fig. 30. *Gila robusta.* Tempe, Arizona. Stanford University.

Fig. 31. *Kneria cameronensis.* Brit. Museum.



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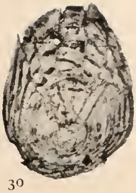

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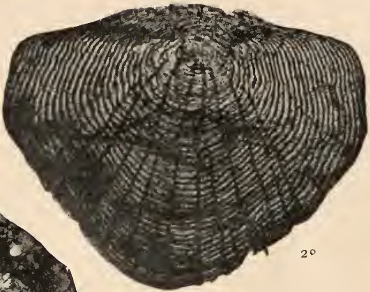

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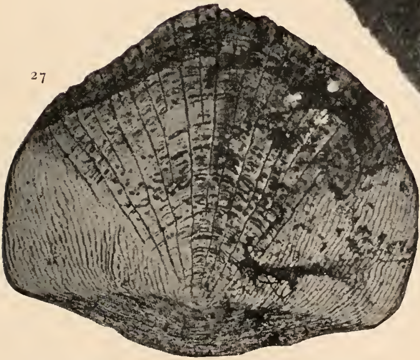

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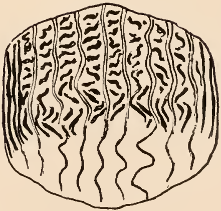

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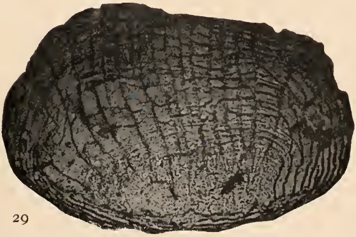

29

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